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U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 100.

B. T. GALLOWAY, *Chief of Bureau.*

MISCELLANEOUS PAPERS.

I. CRANBERRY SPRAYING EXPERIMENTS IN 1905.
By C. L. SHEAR, *Pathologist.*

II. THE WRAPPING OF APPLE GRAFTS AND ITS RELATION TO THE CROWN-GALL DISEASE.
By HERMANN VON SCHRENK, *Special Agent,* and GEORGE G. HEDGCOCK, *Assistant.*

III. GARLICKY WHEAT.
By J. W. T. DUVEL, *Assistant.*

IV. METHODS OF TESTING THE BURNING QUALITY OF CIGAR TOBACCO.
By WIGHTMAN W. GARNER, *Scientific Assistant.*

V. THE DRUG KNOWN AS PINKROOT.
By W. W. STOCKBERGER, *Expert.*

VI. ORCHARD GRASS.
By R. A. OAKLEY, *Assistant Agriculturist.*

VII. THE EFFECT OF COPPER UPON WATER BACTERIA.
By KARL F. KELLERMAN, *Physiologist,* and T. D. BECKWITH, *Scientific Assistant.*

VIII. CONDITIONS AFFECTING LEGUME INOCULATION.
By KARL F. KELLERMAN, *Physiologist,* and T. R. ROBINSON, *Assistant Physiologist.*

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[a] The eight papers constituting this bulletin were issued in separate form on February 7, February 28, April 5, June 9, October 9, October 8, October 20, and November 30, 1906, respectively.

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MISCELLANEOUS PAPERS.

I.—CRANBERRY SPRAYING EXPERIMENTS IN 1905.

By C. L. Shear, *Pathologist.*

INTRODUCTION.

In Farmers' Bulletin No. 221[a] a brief account was given of cranberry diseases, and also the results of spraying experiments with Bordeaux mixture. The results in 1904 were not entirely satisfactory. This was not due, however, to the inefficiency of Bordeaux mixture, but to circumstances which prevented the applications being made at proper intervals. The results obtained in 1904 showed an average of 21.7 per cent of rotten berries on the sprayed plats, as compared with an average of 76.8 per cent rotten on the unsprayed check plats. Considering the unsatisfactory manner in which the Bordeaux mixture was applied, the prediction was ventured that it would be possible with more thorough treatment to reduce the loss from rot to 10 or 15 per cent. The results obtained in 1905 have more than justified this prediction.

The experiments were conducted on what is known as the Bunker Hill bog at Whitesville, N. J., which is in charge of Mr. James D. Holman, the same plats being used as in 1904, with the addition of a small area not heretofore sprayed. This bog was selected because the fruit had in former years been almost entirely destroyed by disease. The water was drained from the bog May 10–12. It is the usual practice of cranberry growers to flood bogs for twenty-four hours during the first week in June, in order to destroy insects. In these experiments it was planned to spray part of the experimental plats before this second flooding and part immediately afterwards, in order to determine the necessity or desirability of spraying before this flooding. The water supply of the bog was, however, insufficient to flood it at the usual time, and it was not done.

The spraying apparatus used was a barrel and force pump fitted with two lengths of half-inch hose, each length provided with an extension rod and two Vermorel nozzles. The apparatus was driven about the bog in a low-bodied spring wagon, as shown in figure 1.

[a] Farmers' Bulletin No. 221, U. S. Dept. of Agriculture, "Fungous Diseases of the Cranberry."

The Bordeaux mixture used consisted of 6 pounds of copper sulphate (bluestone) and 6 pounds of fresh stone lime to 50 gallons of water, to which was also added 4½ pounds of commercial resin-fishoil soap. The addition of this soap has been found to be indispensable, as Bordeaux mixture will not spread over and adhere satisfactorily to the glossy surface of the cranberry leaves and fruit without it. Heretofore this soap has been made as it was needed for use. Its manufacture was not an altogether pleasant operation. Now that the soap is being manufactured and placed on the market at about 3 cents per pound, it is cheaper and much more convenient to purchase it than to make it.

SPRAYING AND ITS RESULTS.

The sprayed plats were numbered 3, 5, 6, 7, and 9, the plats between being left as checks. Plats 3 and 7 were sprayed five times, as follows: May 19, June 22–23, July 14–17, July 31–August 1, and August 15–17. On September 8, accurate counts were made of all the diseased and sound berries on small areas, showing the average condition of the berries on the sprayed plats; also of equal areas, showing the average

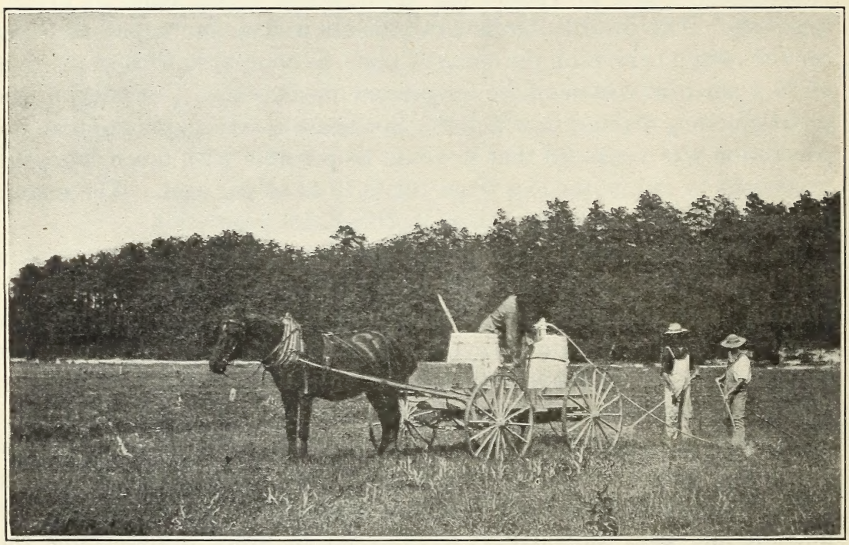

FIG. 1.—The apparatus used in the cranberry spraying experiments.

condition of the berries on the check plats. Plat 3 gave 3.23 per cent of rotten berries, plat 7 gave 8.8 per cent of rotten fruit, check plat 2 showed 91 per cent of rotten fruit, and check plat 8 gave 91.53 per cent of rotten berries, giving an average of a fraction over 6 per cent of rotten fruit for the sprayed plats and a little more than 91 per cent for the unsprayed plats. On these two plats it will be noted that the first application was made on May 19, when the vines had but just com-

menced to put out new growth. This application was the one mentioned as being made before the usual flooding for insects.

Plats 5 and 9 were sprayed five times, as follows: June 2, June 22–23, July 14–17, July 31–August 1, and August 15–17. On June 2, when the first application was made to these plats, there was a good growth of young shoots and leaves. Counts of fruit on small areas, as in the preceding case, gave the following results: Sprayed plat 5, 2.62 per cent of rotten berries; sprayed plat 9, 2.1 per cent of rotten fruit; check plat 4, 91.8 per cent rotten; check plat 10, 93.5 per cent rotten, giving an average of 2.36 per cent of rotten berries on the sprayed plats and 92.6 per cent of rotten fruit on the unsprayed plats. There was very little difference in the amount of rot on this series of plats and that on the series mentioned in the preceding paragraph.

Plat 6 was sprayed but three times, as follows: July 14–17, July 31–August 1, and August 15–17. Counts made, as in the previous cases, on September 8, gave the following results: Sprayed plat, 18.3 per cent of rotten berries; check plat, 91.53 per cent of rotten fruit. It may also be added that a greater number of the berries from this sprayed plat decayed before they were ready for shipment than was the case with the fruit from the plats which received five applications.

The fruit from an area of 1,048 square feet, showing average condition of fruit on sprayed plat 7, was carefully hand picked and produced 3 bushels of sound fruit, which is at the rate of about 125 bushels per acre. The same area from check plat 8, showing the average condition of fruit, gave a scanty peck, or at the rate of about $10\frac{5}{12}$ bushels per acre. In other words, there was twelve times as much sound fruit on the sprayed as on the unsprayed plat, or a saving of over 100 bushels per acre.

Besides our purely experimental plats, several acres upon another cranberry bog known as "Long Swamp" were sprayed by Mr. Holman. One portion was sprayed five times on the following dates: June 6–10, June 26–28, July 18–21, August 2–4, and August 18–19. One part was sprayed only four times. The first plat, which was also sprayed in 1904, was estimated to have from 80 to 100 per cent of the fruit sound on September 15. On the area sprayed only in 1905 it was estimated that from 70 to 90 per cent of the fruit was sound. On the plat which received the first four applications only, the fruit showed somewhat more rot than on the other plats at picking time. The fruit on these plats had in former years been almost entirely destroyed by rot.

IMPORTANCE OF EARLY APPLICATIONS.

The difference in the appearance of the fruit on the sprayed and unsprayed plats was very marked by the middle of July. On the unsprayed plats a large proportion of the fruit was blasted, owing to the

early attack of the scald fungus (Guignardia), while on the sprayed plats but little blasted fruit was to be seen. In many cases at least one-half of the fruit is destroyed by blasting, the young fruits being attacked by the fungus at about the time the blossoms begin to fall. In order to prevent this, one of the applications of Bordeaux mixture should be made immediately after the vines have reached their maximum flowering stage, as a delay of a week at this time may make a difference of from 25 to 50 per cent in the amount of fruit destroyed by blasting.

A very striking illustration of this fact was observed upon another bog where one plat had been sprayed on July 1 and another adjoining was not sprayed until July 8. On the plat sprayed on July 1, when the vines had just reached their maximum flowering condition, but very little blasted fruit could be found, whereas on the plat which had been sprayed on July 8 about one-half of the fruit had been blasted. This and other observations indicate the exceedingly great importance of prompt and thorough early applications of the fungicide. In case the bog to be sprayed is flooded for insects early in June, the first application of Bordeaux mixture should be made within a day or two after the water is removed, the second application just as the plants begin flowering, and the third just after the majority of the blossoms have appeared, which in ordinary seasons will be about the first of July.

EFFECT OF SPRAYING PLANTS WHEN IN FULL BLOOM.

In order to determine whether any injury would result from spraying the plants while in bloom, part of one plat was sprayed when in full bloom, and the amount of fruit which set upon this plat was carefully compared with that on adjoining plats which had not been sprayed. No difference could be noted in the amount of fruit on the sprayed and on the unsprayed plats. In addition, certain bunches of vines were dipped in Bordeaux mixture when in full bloom, but without any apparent injury to the fruit. From our observations and experiments it does not appear that there is much danger of loss from spraying vines while in bloom. What little loss might possibly arise from this cause would be very slight compared with the amount of loss from blasted fruit in case the spraying was delayed too long.

KEEPING QUALITIES OF SPRAYED AND UNSPRAYED FRUIT.

A comparison of the sprayed and unsprayed fruit at the time of picking does not give an exact idea of the amount of profit derived from the treatment, as there was a much greater loss of unsprayed than of sprayed fruit between the time of picking and the time the berries were marketed.

In order to compare the keeping qualities of the sprayed and unsprayed fruit, as well as unsprayed fruit which had been treated

with a solution of copper sulphate (1 part to 1,000 of water), 3,600 berries, perfectly sound so far as could be seen from external appearance, were selected from fruit picked on September 18, 1905. Of these, 1,200 were from the sprayed plats and the remainder from the unsprayed check plats. These berries were kept in glass dishes in the laboratory and counted each week, in order to determine the amount of disease which developed. On October 18, about the time the fruit from the bog was marketed, 9.8 per cent of the sprayed fruit showed diseased berries, while 38.1 per cent of the unsprayed fruit and 37.4 per cent of the unsprayed fruit which had been treated with the copper-sulphate solution were diseased. In other words, four times as much of the unsprayed fruit decayed between the time of picking and marketing as of the sprayed fruit.

Parts of the check plats which were worth picking were picked by hand and the fruit kept separate. This fruit was sorted for shipment on October 15, 1905, when from 85 to 90 per cent of it was rotten. Mr. Holman states that 40 per cent of all the unsprayed berries from Bunker Hill bog decayed between the time of picking and marketing. No treatment of the berries with fungicides after picking is likely to give satisfactory results, as we have found that the decay which occurs in storage does not arise from germs which are on the surface of the fruit at the time it is picked, but apparently from a dormant form of the fungus already within the berries, where it is awaiting favorable conditions for development.

As the time for picking approached, so much of the Bordeaux mixture adhered to the sprayed fruit that it was feared enough might be present when the berries were marketed to interfere with their sale. This, however, did not prove to be the case, as the greater portion of the mixture was removed from the fruit during the processes of picking, sorting, and preparing the fruit for market.

As the result of three years' spraying experiments, it is safe to say that by the proper use of Bordeaux mixture the loss from fungous diseases can be reduced to 10 per cent or less. The loss may be slightly more the first year a badly diseased bog is treated, as the benefit, as shown in these experiments, is greater the second year than the first, and is evident not only in the prevention of scald and rot of the fruit, but in the general improvement, thriftiness, and productiveness of the vines. Bordeaux mixture has also been applied with very beneficial results to young vines not yet in bearing, the leaves of which were badly affected by the scald fungus.

COST AND RECOMMENDATIONS.

The cost of the spraying as it was done in these experiments averaged from $15 to $20 an acre, the mixture being applied at the rate of four

barrels, or 200 gallons, an acre at each application, making for the five applications a total of 1,000 gallons to the acre.

In undertaking the application of Bordeaux mixture it is necessary that all material and apparatus be in perfect readiness before the time to begin the work, and nothing should be allowed to interfere with the application of the fungicide at proper intervals; otherwise, the results will be unsatisfactory, and the remedy is likely to be unjustly condemned.

The necessity for care and thoroughness in the preparation and application of the mixture must also be strongly emphasized. The mixture should be made as described in Farmers' Bulletin No. 221, using only good, fresh stone lime and adding resin-fishoil soap. A good nozzle (the Vermorel type is best) should be used and the vines very thoroughly covered at each spraying. Four barrels of the mixture to the acre are ordinarily sufficient, but where the vines are very dense and heavy five barrels may be necessary. Where there is an excessive growth of vines, it will be found advantageous to rake them with a knife rake and thin them out, as is frequently done in order to prepare a bog for picking with scoops.

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B. P. I.—203.

II.—THE WRAPPING OF APPLE GRAFTS AND ITS RELATION TO THE CROWN-GALL DISEASE.

By Hermann von Schrenk, *Special Agent in Charge of the Mississippi Valley Laboratory,* and George G. Hedgcock, *Assistant in Pathology.*

INTRODUCTION.

The crown-gall disease of apple trees, as indicated in a recent publication of the Bureau of Plant Industry,[a] appears in three distinct types—the hard crown-gall, the soft crown-gall, and the hairy-root forms. While the hairy-root disease of apples has usually been considered a type of the crown-gall disease, it in reality is an entirely different trouble, with manifestations which are not in the least like those met with in the true crown-gall disease. The soft type of crown-gall on the apple has not yet been clearly differentiated from the hard type, and in the following discussion the two are considered as one.

The knots which characterize the crown-gall disease of the apple usually appear at some point, either on the root piece or on the scion, where the two are united; that is, the gall may form at the end of the tongue of either the scion or root piece, or at any point where either piece has been wounded. This fact is one commonly recognized by all nurserymen and scientific workers who have studied this disease. About 90 per cent of these knots will appear on the end of the scion piece. The exact cause of the formation of the gall is as yet somewhat uncertain. It would seem, however, that, whatever the cause, the point most exposed to the disturbing factor, whether it be due to a fungus, to bacteria, or to soil or atmospheric conditions, is the junction of the scion and root piece.

When the newly made grafts are laid away in the grafting cellar either in sawdust, excelsior, moss, or other bedding material, callous tissue begins to form on the cut surfaces of both scion and root piece. This callous tissue from the two pieces will in time fill the intervening spaces between the surfaces of the scion and the root piece, and ultimately the root callus and the scion callus will join. Where the root piece and the scion are of exactly the same size and where they are united so as to fit exactly, a very perfect union will take place. Where

[a] Bulletin No. 90, Part II, Bureau of Plant Industry, U. S. Dept. of Agriculture, "The Crown-Gall and Hairy-Root Diseases of the Apple Tree," 1905.

there is a difference in size between the root piece and the scion, or where they are more or less imperfectly fitted, as is almost always the case in commercial grafts, the callus formed by the scion and the root piece will not always meet along equal planes. In these cases there will be a tendency for the callus forming on any surface of either piece which is not in direct contact with an opposite surface (no matter how small this surface may be) to grow out from this surface, either laterally or down or up, into the air, with nothing to prevent its growing into small lumps, which may reach considerable size. Very frequently the pressure exerted by the growing callus will push the tongue of the graft outward. A thick cushion of callus will then form between the scion and root piece.

The object of all grafting should be to bring about as rapid a union between the two grafted parts as possible, with the smallest possible period during which an open wound is allowed to remain into which disturbing factors may enter. With this in mind, a number of experiments were made during the past year, the specific objects of which were to confine the formation of callus as far as possible to the spaces between the root and scion pieces, thereby bringing about a closer and more effective union and reducing the chances for the entrance of possible disturbing factors.

It is intimated that the formation of the hard and soft types of apple crown-gall may be due either to a proliferation of callous tissue in cases where an uneven union is taking place and where the callus has had a chance to grow out laterally in an unrestricted manner, or to fungi, bacteria, or unfavorable soil or atmospheric conditions. Whatever the cause, the making of a perfect union in the shortest possible time may serve to prevent the trouble almost wholly if the first explanation given be the cause, and at least largely remove the danger of a possible infection if the second explanation is found to hold good.

ACCOUNT OF EXPERIMENTS.

In order to confine the formation of the callus, a number of grafts were wrapped with various materials during the winter of 1904–5. The wrapping idea is no new one, because cloth and paper were used by nurserymen many years ago in connection with various types of root and top grafting. At the present time cloth and paper are used very little, because the system of thread wrapping will give a larger number of grafts in a given period of time. The materials used in our experiments were cloth, thin sheet rubber (dental rubber), and waxed paper. In addition to this, grafts were made with plain thread and waxed thread, and some grafts were wrapped with plain thread and afterwards the whole union was covered with grafting wax. One series of grafts was made without any wrapping of cloth or

thread whatever, the pieces being simply fitted together with care. All grafts made were of the type known as tongue or whip grafts. The work was done by an expert, so that all of the grafts may be considered as especially well made. No. 1 Kansas roots and a selected lot of scions of the following varieties were used: Winesap, York Imperial, Wealthy, Missouri, and Northern Spy. The grafts were made in February, 1905. They were stored in chopped excelsior for about six weeks, were then planted in eight different localities in Missouri, Illinois, Iowa, Nebraska, Kansas, and Arkansas, and given the usual cultivation during the summer. All showed a fair stand in November, 1905. Some of the grafts were dug in November, and December, 1905, one-third of the total number planted in five test plats in four States being dug. The results obtained are given later.

MANNER OF WRAPPING.

The manner in which the wrapping was made and the material used may be briefly described as follows:

Cloth.—The cloth used was a cheap black calico of the poorest grade obtainable in the market. This was torn into strips 1 inch wide and 4 or 5 inches long. After the graft was made, one end of the cloth was dipped for one-half inch into hot melted grafting wax. Starting with the other end, the cloth was then wrapped tightly around the scion and the free waxed end pressed down. This completed the operation.

Rubber.—The rubber used was of a quality similar to dental rubber, which is also frequently used for insulating wires. It was bought in rolls 1 inch wide, and was cut off in such lengths as were necessary to completely envelop the union. The rubber was usually wrapped so as to go around the union twice, and the free end was fastened with rubber cement applied with a brush.

Waxed paper.—Sheets of ordinary unglazed printers' paper were waxed on one side by coating them with hot grafting wax applied with a paint brush. They were then cut into inch strips, 10 to 20 sheets being cut at one time, and these again into strips about 4 or 5 inches long. One paper strip was then wrapped around the union, the waxed side toward the graft, the free end being stuck down by pressing it on.

Plain thread.—The thread used for the ordinary grafts was a machine cotton, No. 9.

Waxed thread.—The thread used was a machine cotton, No. 28. This thread was soaked in hot grafting wax until thoroughly penetrated and was then allowed to drain while hot.

Plain thread with union waxed.—These were ordinary grafts made with plain thread, wrapped as previously described, and then coated with melted grafting wax nearly at the point of hardening.

GRAFTS LEFT UNWRAPPED.

The grafts left without wrapping of any kind were made with special care, so that when the roots and scions were joined they remained firmly united.

RESULTS OF WRAPPING.

After digging the grafts, a careful examination of the various series was made to determine the effect of the wrapping on the union and on the presence or absence of crown-gall formation.

The following table shows the number of apple trees dug, the character of the wrapping, and the number and percentage of smooth trees and rough trees, the latter class including all trees which were found to have crown-gall, irregular callus, or hairy-root formations:

TABLE 1.—*Comparison of smooth trees and rough trees resulting when different methods of grafting were used.*

Kind of wrapping.	Total number.	Smooth trees.		Rough trees.	
		Number.	Per cent.	Number.	Per cent.
Rubber	675	584	86.5	91	13.5
Cloth	709	604	85.1	105	14.9
Waxed paper	671	474	70.6	197	29.4
Plain thread	645	442	68.5	203	31.5
Waxed thread	675	430	63.7	245	36.3
Plain thread with union waxed	402	178	44.2	224	55.8
Unwrapped	569	312	54.8	257	45.2

Two series of facts may be deduced from this table—

(1) The effect of the various kinds of wrapping on the smoothness of the union.

(2) The effect of wrapping on the presence or absence of crown-gall formations.

EFFECT UPON THE UNION.

Some brief notes on the effect of the wrapping on the union follow. By a smooth graft is meant one which shows no uneven or irregular masses of callus. In the smooth grafts all parts are firmly united, so that no ridge or roughness is felt when the union is rubbed with the fingers. The grafts were graded with the greatest care.

Cloth.—When the grafts were planted in the spring the cloth was usually intact. When dug in the autumn it had almost entirely rotted away on all grafts. The union which resulted under the cloth was generally smooth and very even. In many cases it was difficult to detect the original position of the scion and root parts. The cloth had evidently remained in position long enough to thoroughly confine the callus to the spaces between the wounded surfaces.

Rubber.—When the grafts were planted the rubber wrapping was wholly intact. When dug the rubber was still found around the union

in the majority of cases. It had, however, exerted no injurious effect, because, as the young trees had grown in diameter, the rubber had expanded. The roots had frequently punctured the rubber freely. In other words, the rubber had in no way retarded the growth. The unions formed under the rubber were smooth and even; in fact, they were the most perfect of all those formed in the experiment. The rubber covering was practically waterproof. So perfect were the unions that in many cases it was possible to detect the line of demarcation between the root and scion only by the difference in color.

Waxed paper.—When planted in the spring the waxed paper had not rotted, but it had a tendency to become unrolled, due probably to too much oil in the wax. When dug in the autumn the paper had mostly disappeared. The grafts showed many smooth trees, but the percentage was not nearly so large as in the case of those wrapped with rubber or cloth. Attention should be called to the fact that in all grafts where wax was used the wax itself seemed to exert an unfavorable effect on the union, and the writers are inclined to discourage its use wherever it comes into direct contact with wounded surfaces.

Plain thread.—When planted in the spring the plain thread had in many instances entirely rotted away. The grafts when dug showed many callus proliferations. The percentage of smooth grafts was low when compared with rubber or cloth wrappings. The results were about as good as those secured by the use of waxed paper wrapping, and a little better than those obtained with waxed thread.

Waxed thread.—When the grafts were planted the waxed thread was intact. When dug in the autumn the grafts showed from 6 to 8 per cent with knots caused by the pressure exerted by the thread, which had not rotted away because the wax protected it more or less. The percentage of smooth trees aside from this was almost the same as for the plain thread. No perceptible advantage appeared to be gained by the use of waxed thread as compared with plain thread.

Plain thread with union waxed.—At the time of planting it was noted that the wax had in many instances penetrated between the surface of the two pieces. The callus had formed in irregular masses, there being nothing to confine it. It furthermore appeared as if the wax had exerted the unfavorable effect already referred to in connection with the use of waxed paper. When dug the trees in the majority of instances showed thread knots, due no doubt to the fact that the wax had retarded the rotting away of the thread. The unions were usually rough. The unfavorable effect ascribed to the wax was evident not only in the immediate wound areas, but also all along the bark on both scion and root pieces, wherever the wax came in contact with the tissue.

Unwrapped grafts.—The grafts with no wrapping showed the excessive development of callus more than any others. However, many of

the resulting trees when dug in the autumn showed fairly good unions. The grafts as a whole fell below the wrapped ones in the number of smooth trees. There were more deficient trees in this lot, with the possible exception of those waxed over, owing to defective unions and failures to form a union.

EFFECT ON CROWN-GALL FORMATION.

As indicated in the introduction, one of the objects of the experiment was to determine whether by wrapping grafts it would be possible to reduce the number of trees affected with crown-gall. In the following table the total number of trees dug is given; also the kind of wrapping, and the number and percentage of smooth trees, crown-gall trees, and hairy-root trees:

TABLE II.—*Comparison of smooth trees and trees affected with crown-gall and hairy-root, resulting when different methods of grafting were used.*

Kind of wrapping.	Total number.	Smooth trees.		Crown-gall trees.		Hairy-root trees.	
		Number.	Per cent.	Number.	Per cent.	Number.	Per cent.
Rubber	675	584	86.5	65	9.6	26	3.9
Cloth	709	604	85.1	70	9.8	35	5.1
Waxed paper	671	474	70.6	168	25.0	29	4.4
Plain thread	645	442	68.5	130	20.2	73	11.3
Waxed thread	675	430	63.7	182	27.0	63	9.3
Plain thread with union waxed	402	178	44.2	193	48.1	31	7.7
Unwrapped	569	312	54.8	200	35.2	57	10.0

A study of this table will show that the wrapping reduced the number of crown-gall trees very materially, but the results given should be considered as preliminary and the figures as relative rather than absolute. Only a small number of the trees under test have so far been dug. The remainder will be dug after one or two years' growth.

The most effective wrapping, so far as the true crown-gall is concerned, was that made of rubber (86.5 per cent of smooth trees), followed closely by cloth (85.1 per cent of smooth trees). The cloth wrapping, however, shows the highest percentage of smooth trees per 100 grafts planted when not only the crown-gall but also the hairy-root are considered. The difference is very slight, however. The other wrappings show less favorable results, least of all in the case of the grafts wrapped with plain thread and covered with grafting wax (44.2 per cent of smooth trees), followed closely by those with no wrapping whatever (54.8 per cent of smooth trees).

RECOMMENDATIONS.

From the results so far obtained, the use of either cloth or rubber as material for wrapping apple grafts is recommended. Owing to the expense involved in the use of rubber, cloth will be found the most desirable, and in most cases will probably give results fully as satisfac-

tory as rubber. The writers strongly advise against the wrapping of grafts with thread and subsequently waxing the grafts.

SUGGESTION TO NURSERYMEN.

The results obtained from the experimental plats having shown that the wrapping has materially reduced the number of crown-gall and other types of rough trees, it will be very desirable to test these results on a larger scale. The grafts in these experiments were all made with the greatest care, and it would seem probable that the number of smooth trees obtained was therefore larger than might be the case where commercial grafts are made. The latter are usually not fitted with as much care, either with respect to size of scion and root pieces or with regard to an even and close union in the freshly made graft. It is therefore urged that nurserymen generally test the wrapping of their grafts this winter, either with rubber or cloth, after the manner previously described.

Care should be taken in setting out grafts with different kinds of wrapping to treat them in the same manner, that is—

(1) Use the same variety of scion.
(2) Use the same stock of roots.
(3) Make the grafts at the same time.
(4) Plant the grafts on the same date.
(5) Plant in the same field or at least on similar soil.
(6) Cultivate all grafts alike.

Where such tests are made, it is requested that the Bureau of Plant Industry be informed, so that all the experiments may be compared.

It may prove of interest to estimate the total number of smooth and rough trees to the acre which would result from the use of various wrappings, estimating 18,000 grafts to the acre and using as a basis the percentages of such trees obtained in the tests described in these pages. By smooth trees are meant trees free from crown-gall and other diseases; by rough trees, trees affected with true crown-gall, hairy-root, or manifestations of other diseases at or near the point of union. This estimate has been attempted in the following table, which should be regarded only as suggestive, however. The results are averaged from five identical plats in four different States, containing about 9,000 grafts.

TABLE III.—*Estimate of the number of smooth trees and rough trees which can be raised on an acre of land by using various methods of grafting.*

Wrapping.	Smooth trees.	Rough trees.	Total trees.
Rubber	12,600	1,980	14,580
Cloth	12,960	2,340	15,300
Waxed paper	10,260	4,320	14,580
Plain thread	9,540	4,320	13,860
Waxed thread	9,360	5,220	14,580
Plain thread with union waxed	3,780	4,860	8,640
Unwrapped	6,660	5,580	12,240

SUMMARY.

(1) The crown-gall disease of apple trees usually appears at or near the union of the scion and root piece.

(2) The crown-gall disease may be due to an excessive growth of callus, or to an infection of fungi, bacteria, or other agencies at or near the union.

(3) Protection of the graft at the union will serve to induce a better union and may also aid in keeping out disturbing factors.

(4) In making grafts care should be taken to use root and scion pieces of as nearly the same size as possible.

(5) Grafts wrapped with cloth and with rubber yielded 85.1 per cent and 86.5 per cent of smooth trees, respectively.

(6) Ordinary thread grafts yielded 68.5 per cent of smooth trees; plain thread grafts with the union waxed, 44.2 per cent; grafts with no wrapping, 54.8 per cent.

(7) It is recommended that apple grafts be wrapped with cloth or rubber.

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III.—GARLICKY WHEAT.

By J. W. T. Duvel, *Assistant in the Seed Laboratory.*

INTRODUCTION.

Wild garlic (*Allium vineale* L.) was introduced into the United States from Europe considerably more than a century ago. Since its introduction it has made a slow but steady advance, and is now found growing more or less abundantly throughout the greater part of West Virginia, Virginia, Maryland, Delaware, Tennessee, North Carolina, the northern part of South Carolina, the southern part of Pennsylvania, New Jersey, and Connecticut, and locally in almost every State east of the Mississippi River. In all places where it has become well established it is a veritable pest to farmers, millers, grain dealers, and dairymen.

Wild garlic is one of the worst weeds to eradicate after it has once gained a foothold, being propagated by underground bulbs, aerial bulblets, and in some sections by seeds.

WHEAT CONTAINING GARLIC.

The presence of wild garlic in the grain fields of the central eastern States and in other sections where it is locally abundant has caused a very great loss to agriculture. Farmers have been obliged to sell their garlicky wheat at greatly reduced prices, principally because foreign markets will not buy it except at a low price, and millers as a rule refuse to handle it, for they have been able to grind garlicky grain only at a much increased cost. The garlic bulblets gum the rollers, necessitating the stopping of the mills and the washing of the rollers before the grinding can be resumed. The frequency with which the washing must be done depends on the quantity of garlic present. In extreme cases the washing must be repeated every two or three hours, the operation requiring from ten to fifteen minutes for each set of rollers.

Furthermore, flour made from wheat mixed with garlic bulblets is of inferior quality, as bread made from such flour has the garlic odor so disagreeable to most people. This is especially noticeable if the bread is eaten warm. Moreover, on boards of trade, wheat containing garlic bulblets in considerable quantity is graded as "Rejected," and is then sold only on sample. Wheat of this character is generally sold at a price ranging from 20 to 40 per cent lower than No. 2 Red. How-

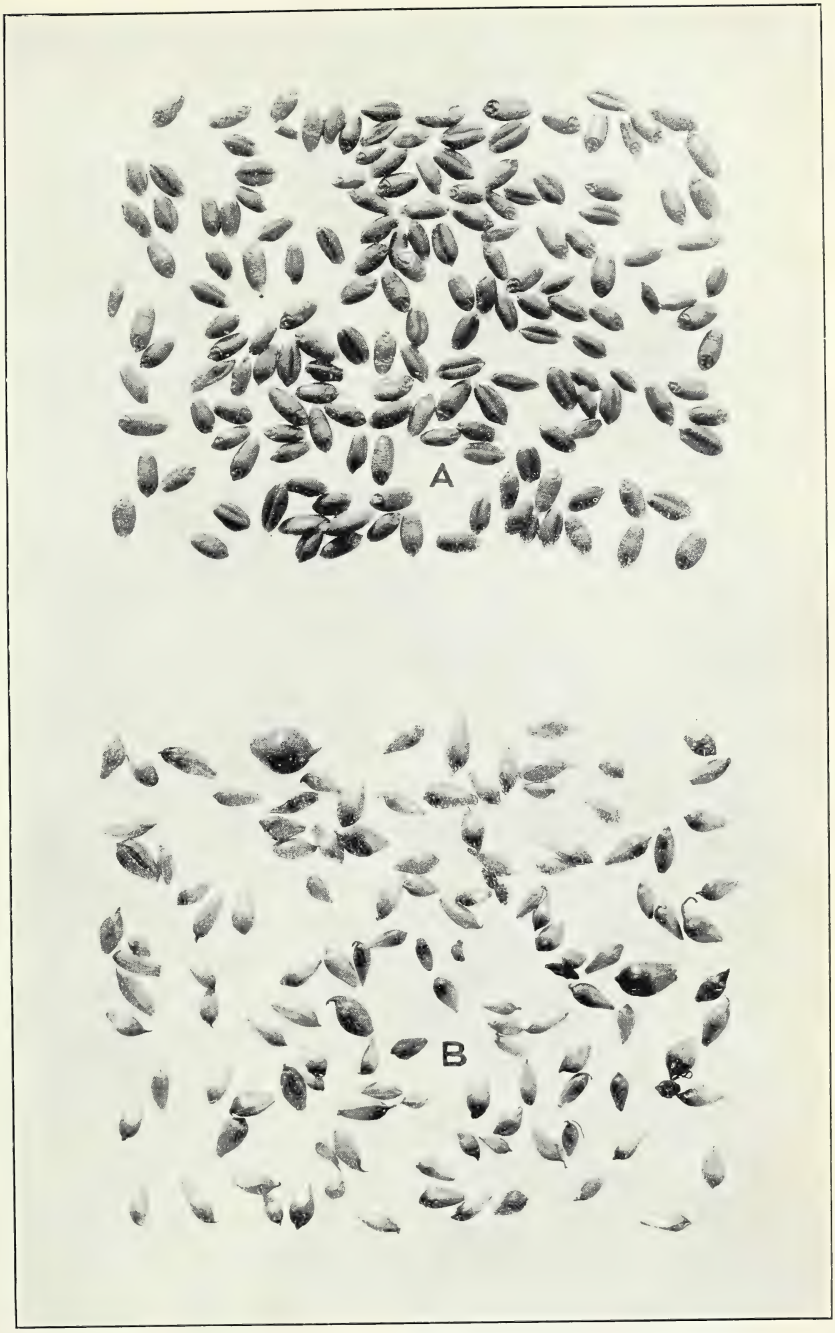
ever, if the garlic bulblets are present only in comparatively small quantity (usually less than one-fourth of 1 per cent) it may pass as No. 2 Red, depending largely on the other foreign substances present and the amount of water in the grain.

At present there are no available data showing definitely the extent of the loss due to the presence of garlic in grain; but in wheat alone this loss is known to be very great. In many sections the growing of wheat has been almost wholly abandoned as a result of the reduced price at which garlicky wheat must be sold. An annual loss of $1,500,000 is undoubtedly a very conservative figure. It has been estimated by members of the Chamber of Commerce of Baltimore that 60 per cent of the wheat grown in that section of the United States contains more or less garlic. The three States in which garlic does the greatest injury to the wheat crop are Maryland, Virginia, and Tennessee. The average yield of wheat from these three States during the five years from 1900 to 1904, inclusive, was just short of 29,000,000 bushels. Allowing that 50 per cent of this wheat contains garlic, we have 14,500,000 bushels of garlicky wheat in these three States alone. But granting that only one-half of this amount contains garlic in sufficient quantity to throw it out of grade, we still have 7,250,000 bushels of wheat which must be sold at a greatly reduced price. A reduction of only 15 cents per bushel means more than a million dollars annually to the farmers of Maryland, Virginia, and Tennessee. The members of a prominent firm of grain exporters in Baltimore state that the depreciation for the Maryland crop alone, which amounts to about 12,000,000 bushels annually, will be fully 5 to 10 cents per bushel, or an equivalent of $600,000 to $1,200,000. A large quantity of garlicky wheat, however, does not get into the elevators, being fit only for feeding purposes. Mr. R. L. Wells[a] states that, in Tennessee, wheat containing garlic bulblets has been sold as low as 15 cents per bushel to feed stock.

EXPERIMENTS IN SEPARATING GARLIC FROM WHEAT.

The presence of the aerial bulblets of wild garlic in wheat has always been objectionable, principally because of the extreme difficulty of separating them from the wheat. While some of the lighter, immature bulblets can be blown out by a good fanning mill, the greater number are of practically the same size and weight as the wheat kernels. Plate I shows wheat kernels and the aerial bulblets of wild garlic of natural size. This similarity in size and shape makes it impossible to separate them during the autumn or early winter by the use of the ordinary cleaning machinery usually found in the majority of flour mills and elevators, i. e., by screening and fanning. If the bulblets

[a] The Wild Onion, Bulletin, Tennessee Agricultural Experiment Station, July, 1895.



WHEAT KERNELS (A) AND THE AERIAL BULBLETS OF WILD GARLIC (B).
(Natural size.)

are allowed to freeze, they afterwards become dry and are then quite readily blown out, but this is not always practicable.

In view of this fact experiments were undertaken in June, 1905, in order to ascertain whether the mixture of garlic and wheat could not be dried artificially, thereby reducing the weight of the bulblets to such an extent that they could be satisfactorily removed as soon as the grain is ready for market. The detailed results of these experiments[a] are given in the following pages.

LOT A.

Lot A consisted of approximately 44 bushels of "rejected" wheat furnished by the Baltimore Chamber of Commerce. When received it contained 16.55 per cent of water and 2.17 per cent of garlic. The amount of foreign seed and chaff present was not determined. The value of this wheat was placed at 65 or 70 cents a bushel.

Experiment No. 1.—A portion of this wheat was dried in the small grain drier of the Seed Laboratory at a maximum temperature of 136° F. for two hours. During this time the moisture content of the grain was reduced from 16.55 per cent to 9.5 per cent, or from 2½ to 4½ per cent less than good American wheat normally contains. But this degree of drying proved insufficient, as 0.28 per cent of garlic still remained in the sample after a preliminary cleaning. This same lot of wheat was therefore dried for an additional half-hour and the moisture content was reduced to 8.94 per cent.

Experiment No. 2.—Another portion of seed from Lot A was dried a few days later for nearly four hours, the maximum temperature reading 140° F. At the termination of the drying a moisture determination of a sample of this wheat showed only 5.87 per cent of water.

The wheat from experiments Nos. 1 and 2 was then mixed and cleaned, and the average percentage of water in the mixed sample was found to be 7.41 per cent. After cleaning, an analysis of this wheat showed that the amount of garlic had been reduced from 2.17 to 0.05 per cent, 97.6 per cent of the garlic having been removed. Plate II, figure 1, shows a 1-pound sample of this wheat as received, the quantity of garlic in 1 pound when received, and the quantity of garlic remaining in 1 pound after drying and cleaning.

Concerning this lot of wheat the secretary of the Baltimore Chamber of Commerce wrote as follows:

> The wheat which you cleaned and returned was the source of a great deal of interesting comment upon the floor of the chamber, and the general idea is that a very vast change was accomplished by running it through the drier. The sample sent originally was of such low

[a] Acknowledgments are due to the members of the Baltimore Chamber of Commerce and to Mr. Walter Roberts, of Alexandria, Va., and Mr. E. H. Darby, of Seneca, Md., who kindly supplied the garlicky wheat for these experiments.

and inferior grade as to prohibit it from going into the elevators, and the drying and cleaning to which it was subjected made it No. 2 Red, the contract grade, a difference in value of fully 17 cents per bushel.

An increase of 17 cents per bushel was equivalent to 24.6 per cent of the value of this wheat before drying and cleaning.

LOT B.

A second sample of approximately 38 bushels of "rejected" wheat furnished by Mr. Walter Roberts, Alexandria, Va., contained 0.56 per cent of garlic and 15.08 per cent of water and weighed only 57.5 pounds per bushel.

The lot was divided into three parts for treatment, as follows:

Experiment No. 3.—In this test the drying was continued for three hours, the temperature of the air varying from 153° to 158° F. and the temperature of the wheat from 117° to 155° F. The moisture was reduced from 15.08 to 7.92 per cent. The weight per bushel was increased from 57.5 to 59.25 pounds on drying and to 60.6 pounds after cleaning. The quantity of garlic was reduced to 0.05 per cent, the same as the combined results of experiments Nos. 1 and 2.

Experiment No. 4.—The period of drying in this experiment extended over three and one-half hours, the temperature of the air being the same as in experiment No. 3, 153° to 158° F., the temperature of the wheat gradually increasing from 95° F. at the end of the first half-hour to 145° F. During the three and one-half hours' drying, the water content of the wheat was reduced to 6.88 per cent and the weight per bushel increased to 59.5 pounds. After cleaning, the weight per bushel was increased to 60.7 pounds and the quantity of garlic reduced from 0.56 per cent to 0.06 per cent. Plate II, figure 2, shows a 1-pound sample of this wheat as received, the amount of garlic in 1 pound when received, and the amount of garlic remaining in 1 pound after drying for three and one-half hours and cleaning.

Experiment No. 5.—The last portion of Lot B was dried for two and three-fourths hours, the temperature of the wheat reaching 122° F. in three-quarters of an hour, and 138° F. after one hour, which temperature was maintained for one-half hour, gradually decreasing during the last one and one-quarter hours to 117° F., when the experiment was concluded. The moisture content of the wheat was reduced from 15.08 to 8.48 per cent and the weight per bushel raised from 57.5 to 58.6 pounds. After cleaning, the weight per bushel was 60 pounds and the garlic present 0.07 per cent.

After drying and cleaning, the wheat from Lot B graded No. 2 Red, having at that time a value of 85 cents per bushel. As in its original condition the wheat was purchased for 55 cents per bushel, the drying and cleaning increased its value 54.5 per cent.

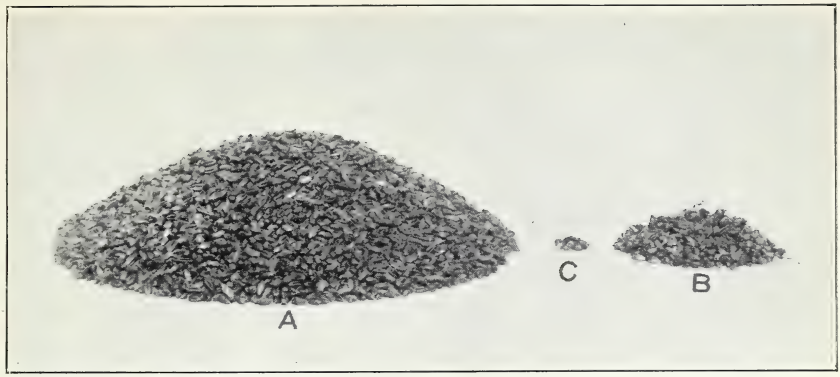


FIG. 1.—WHEAT AND GARLIC FROM LOT A.

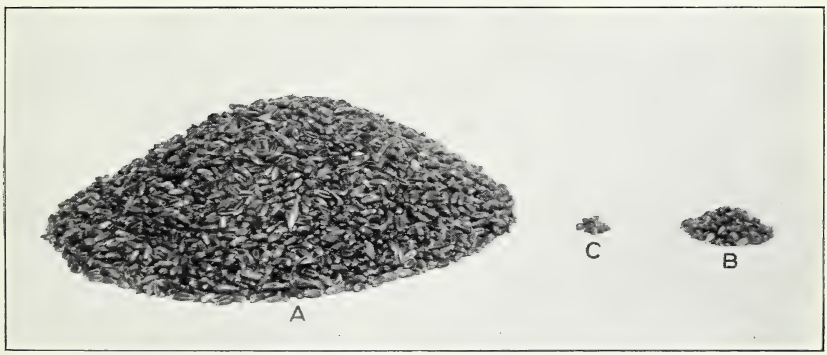


FIG. 2.—WHEAT AND GARLIC FROM LOT B.

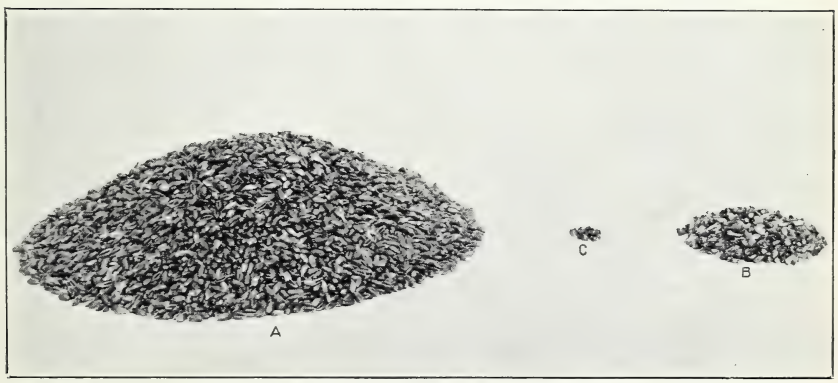


FIG. 3.—WHEAT AND GARLIC FROM LOT C.

ONE-POUND SAMPLES OF GARLICKY WHEAT AS RECEIVED (A, A, A); THE QUANTITY OF GARLIC IN ONE-POUND SAMPLES WHEN RECEIVED (B, B, B); THE AMOUNT OF GARLIC REMAINING IN ONE-POUND SAMPLES AFTER DRYING AND CLEANING (C, C, C).

LOT C.

A consignment of approximately 30 bushels of "rejected" wheat, containing 2.04 per cent of garlic, 16.2 per cent of water, and weighing only 56.5 pounds a bushel, was lent to the Department of Agriculture by Mr. E. H. Darby, of Seneca, Md.

This lot of wheat was divided into two parts and treated as experiments Nos. 6 and 7.

Experiment No. 6.—This wheat was subjected to an air temperature of 113° F. for one hour and of 154° F. for two hours, the maximum temperature of the grain for the last half hour being 149° F. The moisture content was reduced from 16.2 per cent to 8.2 per cent. The weight per bushel was raised to 57.8 pounds after drying and 60.6 pounds after cleaning, and the amount of garlic was reduced to 0.17 per cent.

Experiment No. 7.—This experiment continued for three hours, as in experiment No. 6, but the temperature of the air current decreased gradually from 146° to 122° F., the maximum temperature of the grain being 131° F. Samples taken at the termination of the experiment showed a moisture content of 8.83 per cent. The weight per bushel was increased to 57.5 pounds after drying and 60.2 pounds after cleaning. Plate II, figure 3, shows a 1-pound sample of this wheat as received, the amount of garlic in 1 pound when received, and the amount of garlic remaining in 1 pound after drying for three hours and cleaning.

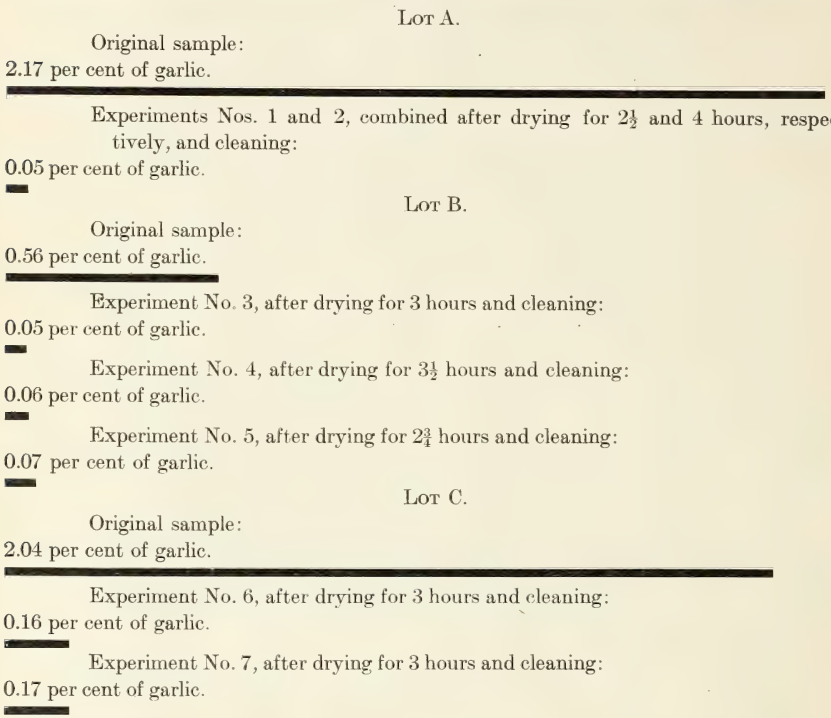
A sample of this cleaned wheat was examined by the chief inspector of the Baltimore Chamber of Commerce and graded as No. 2 Red, giving it a value of 84.5 cents per bushel. The highest price offered for the original lot of wheat was 60 cents per bushel. The removing of the garlic and the cleaning consequently enhanced the value 40.8 per cent.

In experiments Nos. 6 and 7 the drying was not continued quite long enough for the best results, although the quality of the wheat was raised to "contract" grade. At temperatures from 150° to 158° F. the drying should continue for two and one-half to three hours, or until the moisture content of the wheat is reduced to about 8 per cent.

In none of the experiments was it possible to remove all of the garlic, but in every case the quantity was reduced considerably more than was necessary to make the wheat grade as No. 2 Red. Moreover, the quantity of garlic present after the cleaning was not considered sufficient to interfere with the milling of the wheat or to injure the quality of the flour.

The following diagrammatic figures show the relative quantity of garlic in the wheat before and after treatment:

Percentages, by weight, of garlic in wheat, Lots A, B, and C, before and after drying and cleaning.


Lot A.
Original sample:
2.17 per cent of garlic.
Experiments Nos. 1 and 2, combined after drying for 2½ and 4 hours, respectively, and cleaning:
0.05 per cent of garlic.
Lot B.
Original sample:
0.56 per cent of garlic.
Experiment No. 3, after drying for 3 hours and cleaning:
0.05 per cent of garlic.
Experiment No. 4, after drying for 3½ hours and cleaning:
0.06 per cent of garlic.
Experiment No. 5, after drying for 2¾ hours and cleaning:
0.07 per cent of garlic.
Lot C.
Original sample:
2.04 per cent of garlic.
Experiment No. 6, after drying for 3 hours and cleaning:
0.16 per cent of garlic.
Experiment No. 7, after drying for 3 hours and cleaning:
0.17 per cent of garlic.

Garlic bulblets as found in wheat contain from 35 to 70 per cent of water, while the water content of fresh garlicky wheat usually varies from 15 to 20 per cent. During the drying the amount of water in the wheat is decreased, but at the same time the kernels become more compact and the specific gravity is increased, as is shown by the weight per bushel before and after drying. On the other hand, the specific gravity of the garlic bulblets is lowered by the drying. The outer membranous coverings of the bulblets remain distended and the shrinkage takes place in the inner portion, thus leaving a small air space between the bulb proper and the outer protecting layers. This increased air space, together with the decreased weight due to the loss of water, makes it possible to separate most of the bulblets from the wheat by ordinary cleaning machinery.

THE TÓTAL COST OF DRYING AND CLEANING GARLICKY WHEAT.

The total cost, including the shrinkage, of drying and cleaning any given lot of wheat for the removal of garlic depends on four factors: (1) The amount of garlic removed; (2) the amount of chaff and other

foreign substances, aside from the garlic, removed; (3) the percentage of water removed from the wheat; (4) the cost of operating the machinery.

The amount of garlic removed.—In the experiments with the three lots of wheat herein described practically all of the garlic was removed, and this must be considered as a loss in weight. The average loss for each of the three lots of wheat due to the removal of garlic was 2.12 per cent, 0.50 per cent, and 1.88 per cent for Lots A, B, and C, respectively.

The amount of chaff and other foreign substances, aside from the garlic, removed.—The loss in weight due to the cleaning, aside from the quantity of garlic, depends entirely upon the amount of light, immature wheat, chaff, and other foreign substances removed. This loss bears the same ratio for any lot of wheat. Consequently, strictly speaking, this additional decrease in weight can not be considered as an extra expense in the treatment of garlicky wheat. Moreover, the quantity of foreign substances present has an important bearing on the grading of the grain.

The following summary shows the percentages of screenings, including the garlic, obtained from the wheat treated as experiments Nos. 3, 4, 5, 6, and 7:

Experiment No. 3 gave 611 pounds of clean wheat and 28 pounds, or 4.4 per cent, of screenings. Experiment No. 4 gave 548.5 pounds of clean wheat and 15.5 pounds, or 2.8 per cent, of screenings. Experiment No. 5 gave 736.5 pounds of clean wheat and 26.5 pounds, or 3.4 per cent, of screenings. The average percentage of screenings from experiments Nos. 3, 4, and 5 (Lot B) was 3.54 per cent. Deducting from this the amount of garlic removed from Lot B, 0.50 per cent, there is left 3.03 per cent, the proportion of immature wheat, chaff, and other foreign substances removed.

In experiments Nos. 6 and 7, 1,536 pounds of dried wheat gave 135 pounds of screenings, an equivalent of 8.8 per cent, of which 1.88 per cent was garlic, leaving 6.92 per cent of immature wheat, chaff, and other foreign substances removed.

The percentage of water removed from the wheat.—Garlicky wheat almost invariably contains a high percentage of water, and the greatest loss in weight is probably due to the liberation of water during the drying process. In these experiments the quantity of water was reduced from 16.55 per cent, 15.08 per cent, and 16.20 per cent to an average of 7.41 per cent, 7.76 per cent, and 8.52 per cent for Lots A, B, and C, respectively. In order that the garlic may be removed satisfactorily it is necessary to reduce the water content to approximately 8 per cent, which is from 4 to 6 per cent less than No. 2 Red wheat normally contains. However, the dried wheat will again absorb water from the atmosphere, and after the lapse of a few days the water content will be

practically the same as that of air-dried wheat. Likewise the clean, dried wheat can be mixed with any garlic-free lot of wet wheat and the grade of the latter improved in this way. For this reason, only the difference between the water content of the wet garlicky wheat and that of No. 2 Red, which averages about 13 per cent during the first few months after harvesting, should be considered as actual loss in weight due to drying. On this basis the loss due to the removal of water was 3.55 per cent for Lot A, 2.08 per cent for Lot B, and 3.20 per cent for Lot C.

The cost of operating the machinery.—The cost of the actual drying and cleaning alone is very small. With the low pressure boilers available for use with the small grain drier in the Bureau of Plant Industry the maximum temperature possible is only 158° F. At this temperature it is necessary to continue the drying for from two and one-half to three hours in order that the weight of the garlic may be sufficiently reduced so that it can be removed. With the high pressure boilers such as are found in most grain elevators and flour mills an air temperature of 170° to 180° F. can be readily maintained, at which temperature the time factor can be greatly reduced. By careful calculation it is believed that the actual cost of operating the machinery for the drying and cleaning should not exceed one-half cent per bushel.

This factor, however, will vary with the capacity of the drier and the number and size of the other kinds of machinery being operated simultaneously by the same boilers.

THE NET COST OF REMOVING GARLIC.

To ascertain the net cost of removing garlic bulblets from wheat in order to bring it up to "contract" grade, only the following items need be taken into consideration: (1) The cost of operating the machinery; (2) the loss in weight due to the quantity of garlic actually removed, and (3) the difference in the amount of water normally contained in good air-dried wheat, which is not far from 13 per cent, and the amount of water in the garlicky wheat before it goes into the drier. On this basis the cost of drying and cleaning the garlicky wheat discussed in the foregoing pages was 6.3 per cent, 3.2 per cent, and 5.7 per cent, or an equivalent of $5\frac{1}{2}$ cents, $2\frac{3}{4}$ cents, and $4\frac{4}{5}$ cents per bushel for lots A, B, and C, respectively, as governed by the prices current at that time.

THE EFFECT OF THE DRYING ON THE MILLING QUALITIES OF THE GRAIN.

No flour was made from any of the wheat after drying and cleaning; but the consensus of opinion of the majority of the millers to whom samples of the dried wheat were submitted was that the milling qualities of the wheat had not been injured by the drying. Such wheat,

however, is not fit for milling until it has absorbed water from the atmosphere, or has been mixed with damp grain, or steamed, in order to toughen the bran. If the milling is attempted while the wheat is exceptionally dry, the bran will be easily broken, resulting in the production of coarse, dark flour.

THE EFFECT OF THE DRYING ON THE VITALITY OF THE WHEAT.

The objection has frequently been made that the high temperature ordinarily used in the commercial drying of grain will destroy the germinating power. In the majority of cases the vitality of the grain after drying is of little importance, as such grain is seldom used for sowing or planting. The foregoing objections, however, are not well established, as the vitality of grain is not injured by drying in commercial grain driers at the temperatures commonly employed.

The grounds for the belief that temperatures as high as 140° to 175° F. for periods of short duration will destroy the vitality of grains are based on laboratory tests in which no provision was made for the circulation of air. Under such conditions the life-giving principles are readily destroyed, especially when considerable moisture is present. But when the drying is done in such a way that the moisture liberated will be readily carried away, as in commercial grain driers, there is little danger of destroying the vitality of the grains, even though the duration of drying be several times greater than that given for the foregoing experiments.

The following table shows the effect of the drying on the germinating capacity of the samples of garlicky wheat from lots B and C, already discussed:

Percentages of germination of wheat from lots B and C before and after drying.

Sample mark.	Duration of drying.	Temperature of air current in drying.	Maximum temperature of wheat.	Water content of wheat.	Germination.
	Hours.	*Degrees F.*	*Degrees F.*	*Per cent.*	*Per cent.*
Original sample				15.08	80
Experiment No. 3	3	153–158	155	7.92	83.5
Experiment No. 4	3½	153–158	145	6.88	85
Experiment No. 5	2¾	155–108	138	8.48	79.5
Original sample				16.20	82
Experiment No. 6	2½	113–154	149	8.20	83
Experiment No. 7	3	146–122	131	8.83	85

With but a single exception the percentages of germination were higher after the drying than before, and such is generally true. In all cases the germination was low, due to the damaged condition of the grain when received.

While the tests made are few in number, the results given in the foregoing table are sufficient to show that a good quality of garlicky wheat can be dried and afterwards cleaned and used for sowing with entirely satisfactory results. The garlic bulblets, as found in wheat,

contain from 35 to 70 per cent of water. With this high percentage of water the greater quantity of the bulblets are partially cooked or scalded during the drying process, thus rendering growth impossible.

MACHINERY USED FOR DRYING AND CLEANING.

The drying was done in a small grain drier. In the absence of a good fanning and screening machine for the larger grains, the wheat was first run through a fanning mill specially constructed for cleaning clovers, alfalfa, and timothy. The greater quantity of the garlic was blown out, but many of the larger bulblets could not be removed in the absence of screens, and for this reason the wheat, for the final cleaning, was put through a "shaker" such as is commonly used for cleaning rice.

It is not desired to place any special emphasis on the particular machinery used for these experiments. Any of the good commercial driers with any good cleaning machinery should give satisfactory results.

SUMMARY.

The presence of the aerial bulblets of wild garlic in a large quantity of the wheat grown in the central eastern United States causes a great depreciation in its value. The loss to agriculture from this cause alone is very conservatively estimated at more than $1,500,000 annually.

The wheat kernels and the garlic bulblets are very similar in size and weight, which makes their separation by the methods ordinarily in use next to impossible as long as they are fresh.

If wheat containing garlic is artificially dried, the wheat kernels increase in specific gravity and the garlic bulblets decrease in specific gravity, so that practically all of the latter can be removed by good cleaning machinery.

Garlicky wheat is usually wet, often containing as much as 20 per cent of water, and the drying should be continued until the moisture is reduced to approximately 8 per cent.

In estimating the total cost of the treatment of a lot of garlicky wheat, only the amount of garlic removed, the excess of moisture above that which good No. 2 Red wheat usually contains, and the cost of operating the machinery need be considered. The cost of removing the chaff, immature wheat, etc., is the same as for the cleaning of any sample of wheat free from garlic.

The commercial drying of wheat in a good commercial grain drier does not injure its vitality, while most of the garlic bulblets are killed, owing to the higher percentage of water in the latter.

It has not been definitely determined, but the more general opinion is that the drying does not injure the milling qualities of the wheat.

Any of the good commercial grain driers, together with any good wheat-cleaning machinery, should give satisfactory results.

B. P. I.—212.

IV.—METHODS OF TESTING THE BURNING QUALITY OF CIGAR TOBACCO.[a]

By Wightman W. Garner, *Scientific Assistant, Plant Breeding Investigations.*

INTRODUCTION.

As has been pointed out in previous publications of the Bureau of Plant Industry, a systematic effort is being made to improve the quality and yield of the tobacco crop by employing the latest and most approved methods of selection in the old varieties and by creating and establishing new strains possessing to a marked degree those characteristics most to be desired in the various classes of tobacco which the market demands. This work necessitates the careful testing of a large number of types, as well as many individual selections from each of these types, and for this reason it is very desirable to have at our command methods capable of showing with certainty even slight differences in the essential qualities of the various samples to be examined. It is our purpose to make a careful study of the subject of testing tobacco from a practical standpoint, as well as the relation of the chemical composition of the leaf to its good and bad qualities.

In judging the merits of a cigar tobacco, due regard must be had for the particular use for which it is intended, since the finished cigar consists of three distinct components—the filler, the binder, and the wrapper—each of which must possess certain characteristics. The

[a] In the tobacco breeding experiments conducted by the Plant Breeding Investigations of the Bureau of Plant Industry, particular attention is being given to the improvement of cigar tobaccos, including specially high-grade wrapper and filler types. In connection with these experiments, which are being conducted by Messrs. A. D. Shamel and W. W. Cobey, of this office, it has been found necessary to compare the characters of a large number of selected individual plants to determine which ones are superior in their important characters. The means and methods heretofore used in making such comparative tests were very imperfect, and one important preliminary part of the work is to devise special pieces of apparatus which will enable accurate tests to be made. The devices described by Dr. Garner in the present paper it is believed will greatly facilitate such testing and add to the accuracy of the results. The preliminary notes given by Dr. Garner on the influence of wrapper, binder, and filler on the "burn" of cigars open up an important field of investigation in connection with the testing and breeding of different types of tobacco.—Herbert J. Webber, *Physiologist in Charge of Plant Breeding Investigations.*

filler must have, above all else, a fine flavor and aroma and a good "burn." In the case of the wrapper leaf there are a number of requirements to be met, among which are sufficient elasticity, proper color, size, and shape, fineness of veins, freedom from objectionable flavor and taste, a fine "grain," and a good burn. Many of these qualities can be determined by simple inspection, without the use of any specific tests, while others require special laboratory methods. The present article has to do only with the practical methods of testing the burn, deferring to a later day a consideration of the chemical characteristics of the tobaccos which have been tested.

There are several elements which go to make a good or bad burn, chief of which are the capacity for holding fire, the evenness of the burn, the color of the ash and its firmness, the coaling or carbonization, and the "puckering" of the leaf immediately in advance of the burning zone of the cigar. The final test of any cigar tobacco must, of course, rest in the smoking of the manufactured cigar, but, while this gives a direct means of determining the character of the ash, it does not furnish accurate information as to the evenness of the burn or the fire-holding capacity of any one of the components of the cigar except with reference to the other two particular components used in the experiment. This is particularly true of the wrapper, as was shown by special experiments carried out to observe the effect of using different fillers and binders with the same wrapper. The result of these experiments will be more fully discussed below. Again, it should be remembered, in this connection, that cigars made by the same workman and from the same lot of tobacco often vary widely in their burn owing to the impossibility of avoiding unevenness in the filler, and this source of error can only be eliminated by several times repeating the experiment. It is evident, therefore, that, in order to get reliable data concerning the relative merits of different wrappers with respect to their burning qualities, the cigar test must be supplemented by some other method capable of giving sharp distinctions as to the fire-holding capacity and evenness of burn.

A method which has long been in use is to ignite the leaf by means of a lighted cigar or a slow-burning match devised by Nessler,[a] and note the number of seconds during which it continues to glow. The mean of several tests is taken as a measure of the capacity for holding fire; but the variation in the results obtained, even upon a single leaf, is so great that little reliance can be placed upon the figures except in a very general way. In this method no account is taken of the area of the leaf burned, and the wide differences obtained on a single leaf are due principally to the fact that frequently the ignited zone soon ceases to glow except for one or more very small streamers, which

[a] Landw. Vers. Stat., XI, 399.

continue to burn for a much longer period, thereby giving results altogether out of proportion to the true burning qualities of the tobacco in question. Another serious objection to the method is found in the interference of the veins of the leaf; for it seldom happens that the glow can cross these veins except around the outer edges of the leaf, while in the cigar the veins always run longitudinally and so do not interfere with the burn.

The rational procedure would seem to be to test the burn of the leaf when wrapped in some such form as is actually found on the cigar, but without the use of binder or filler. We have devised a method of this kind in which the leaf, after being properly wrapped and dried, is burned with the aid of a slow current of air. The current of air compensates in a measure for the absence of the filler and binder, while its use obviates the unavoidable irregularities of the latter. A detailed description of the apparatus used and the method of carrying out the operation will be found on page 37. This test, combined with the smoking of the cigar, has enabled us to accurately classify a large number of samples of wrapper leaf with respect to their burning qualities.

THE SMOKING TEST.

It is evident that no two persons would smoke a cigar in exactly the same way, nor would the same individual smoke two cigars under exactly similar conditions. It is necessary, therefore, to use some means of smoking the cigars artificially in order to eliminate the personal equation and secure uniformity of conditions. Dr. E. H. Jenkins, in the Annual Report of the Connecticut Agricultural Experiment Station for 1892, has described an apparatus for smoking cigars which was devised by Mr. S. L. Penfield, of Yale University. The "pull" on the cigar is secured by means of an aspirator which is filled by a continuous inflow of water and emptied at regular intervals by a siphon. We have modified this apparatus in a number of details in order to adapt it to our needs, and we give herewith a description of the form and dimensions which we have finally adopted for use in our investigations. In this apparatus as many as four cigars may be smoked simultaneously, while held in such a position that they may be readily compared throughout the operation.

By reference to the accompanying illustration (fig. 2) it will be seen that the holders (*a*, *b*, *c*, and *d*) for the cigars are so arranged that they all lie in the same vertical plane, each one 2 inches above and having its horizontal arm 2 inches shorter than the next lower. A screen with a white covering is placed immediately in the rear of the holders to serve as a background, thereby facilitating observation of the character of the ash. Between the flask bearing the holders and the aspirator and connected with these by means of glass tubing is a

check valve consisting of a T-tube (*e*), the lower arm of which dips beneath the surface of the water in a suitable vessel (*f*), thus preventing a backward draft through the cigars while the aspirator is filling. The smoke which escapes through this valve has a very disagreeable odor; hence, it is well to use a bottle fitted with a stopper with two holes, through one of which passes the lower arm of the T-tube (*g*), while through the other passes one end of a long tube (*h*), which carries away the obnoxious fumes. The aspirator consists of a glass cylinder (*i*), the upper end of which is fitted with a rubber stopper

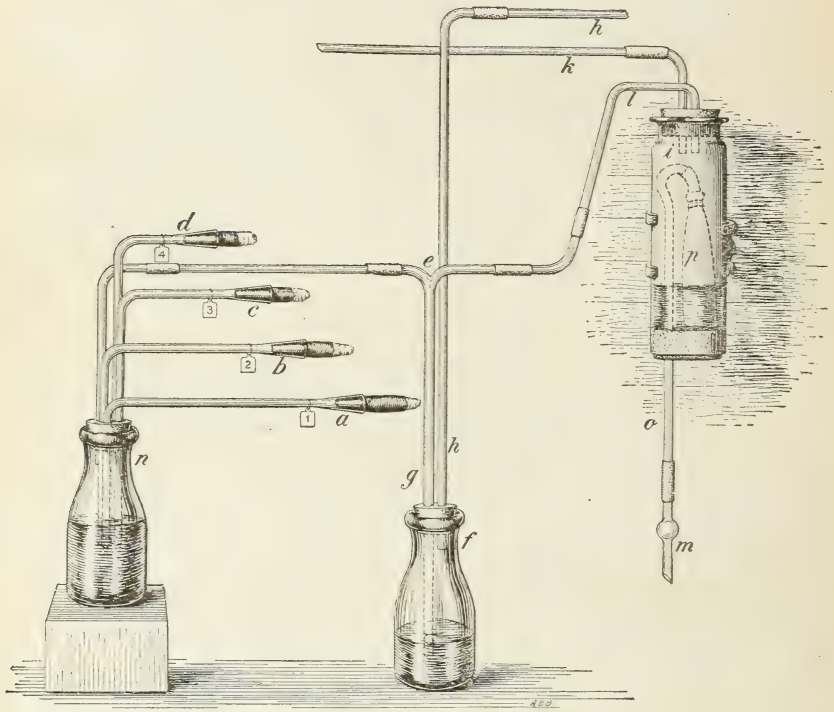
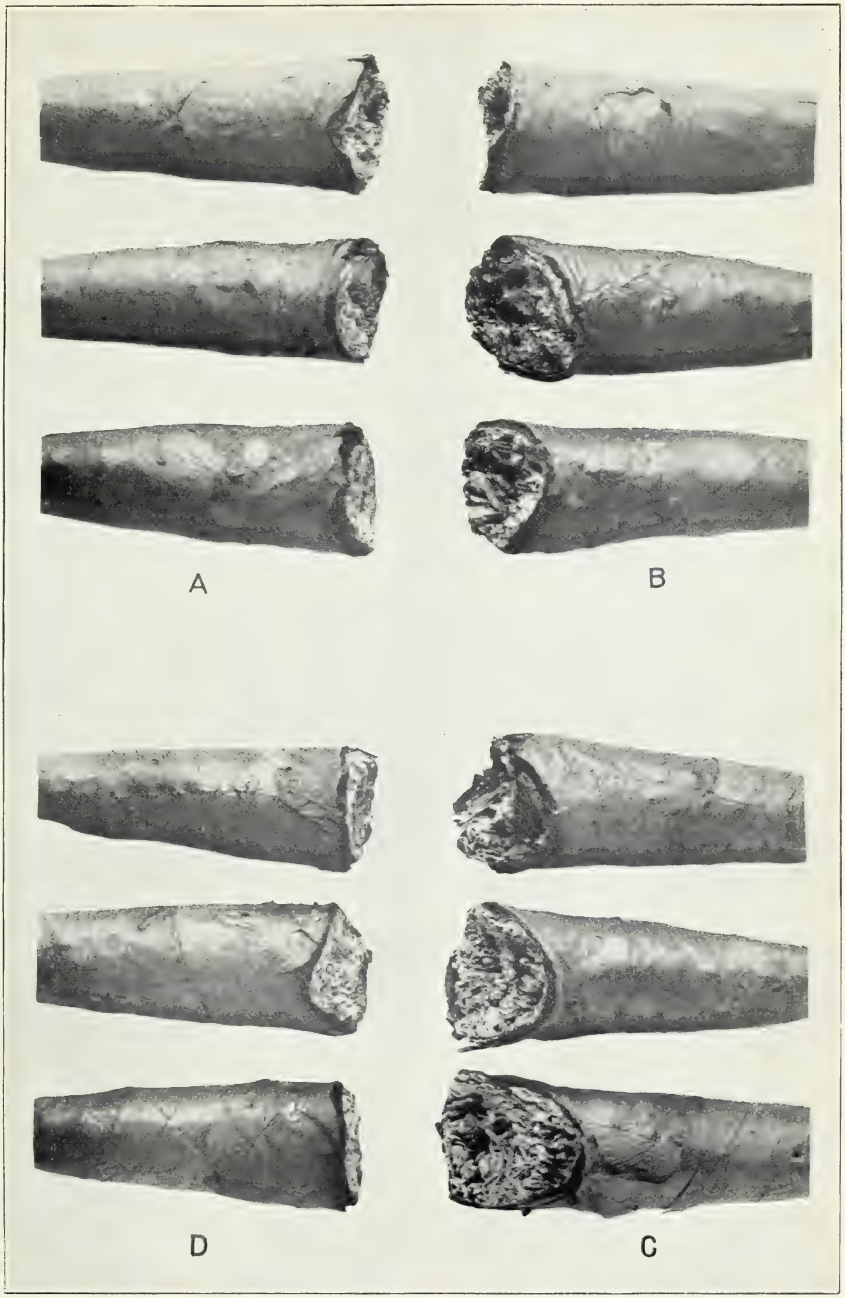


FIG. 2.—Apparatus for testing the burning quality of cigars: *a*, *b*, *c*, *d*, holders for cigars; *e*, T-tube, the lower arm of which, *g*, dips beneath surface of water in *f*; *h*, *h*, tube for leading away tobacco smoke; *i*, aspirator; *k*, tube leading to water supply; *o*, *p*, long and short arms of siphon; *l*, tube connecting cigar holders with aspirator; *m*, tube, with bulb, attached to long arm of siphon; *n*, flask carrying the cigar holders.

containing two holes through which pass tubes leading to the water supply (*k*) and to the flask carrying the cigar holders (*l*), respectively. The lower end of the cylinder is closed with a rubber stopper bearing the small arm of the siphon (*o*). The tube furnishing the water supply is connected with a reservoir provided with a constant-level attachment. To the lower end of the small arm of the siphon is attached a glass tube (*m*) bearing a small bulb which materially assists in breaking the flow of the water at the moment the aspirator is emptied. The tubing connecting the parts of the apparatus should not be less than



VARIATION IN BURN OF WRAPPERS DUE TO DIFFERENT FILLERS: A, FILLER GROWN IN TEXAS FROM CUBAN SEED; B, FILLER GROWN IN OHIO FROM DOMESTIC SEED; C, FILLER GROWN IN SOUTH CAROLINA FROM CUBAN SEED; D, AN IMPORTED CUBAN FILLER.

The same sample of Sumatra wrapper was used throughout and the binder was taken from the same leaf as the wrapper in each case.

6 to 8 mm. internal diameter; otherwise the tubes will frequently become clogged by the condensation products of the smoke. The container for the cigar holders (*n*) is filled about two-thirds full with dilute sulphuric acid, which serves as an acid wash for the smoke, retaining the organic bases and thereby further helping to prevent the choking up of the machine. The lower ends of all the cigar holders should, of course, extend to exactly the same depth below the surface of the acid.

In the machine which we now have in operation, the relation between the short arm of the siphon and the internal diameter of the aspirator is such that the volume of water delivered at each emptying of the latter is 600 c.c. This corresponds to an actual capacity of about 450 c.c. for the aspirator, the difference, of course, representing the volume of water entering the aspirator from the supply pipe while the siphon is in action. The rate of inflow from the supply tank is approximately 900 c. c. per minute. The internal diameter of the long arm of the siphon is 8 mm., while that of the short arm is 25 mm. The entire length of the long arm of the siphon exceeds that of the short arm by 40 cm. An apparatus of the above-mentioned dimensions will smoke four cigars of the Perfecto type, $4\frac{5}{8}$ inches in length, in about thirty minutes, a rate which is probably somewhat above that of the average smoker. The pull on the cigar occurs at intervals of thirty seconds and continues for a period of ten seconds. The frequency of the pull is controlled by the rate of inflow of the water from the supply tank, while its duration is governed principally by the relation between the diameter of the small arm of the siphon and the volume of the aspirator.

THE EFFECTS OF THE FILLER, THE BINDER, AND THE WRAPPER ON THE BURN OF THE CIGAR.

As preliminary to the use of the cigar test in examining wrapper leaf, a series of experiments was carried out to determine the relative effects of the three components of the cigar on the burn. For this purpose a number of cigars were made by an expert workman, using four different types of wrapper on each of four different types of filler. In a portion of the cigars the binder used was taken from the same leaf as the wrapper, while in the remainder a sample of Connecticut Broadleaf tobacco was employed for this purpose. These cigars were smoked in the above-described apparatus under conditions as nearly uniform as could be obtained, and the evenness of the burn and the character of the ash were carefully noted.

With reference to the evenness of the burn, markedly different results were obtained when wrappers taken from the same sample were smoked on different types of filler. A typical case of this kind is shown in Plate III. The twelve cigars shown were all made from the same

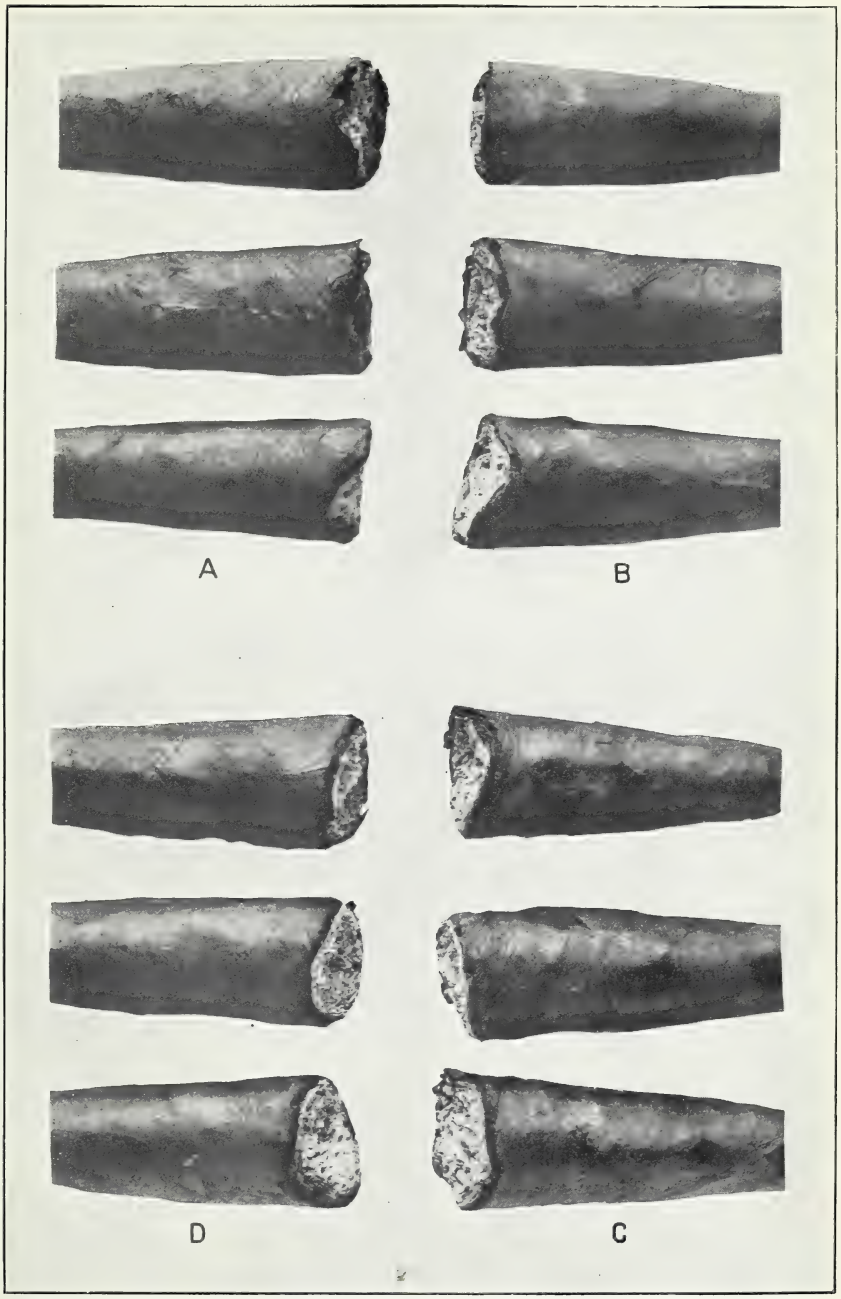
sample of wrapper, and in each case the binder was taken from the same leaf as the wrapper. In the first group (A), a sample of filler grown in Texas from Cuban seed was used; in the second group (B), a heavy filler grown in Ohio from domestic seed; in the third group (C), a filler grown in South Carolina from Cuban seed; in the fourth group (D), an imported Cuban filler. The wrapper used on these cigars was a type of Sumatra tobacco grown in Connecticut and had a very good burn. The Texas and imported Cuban fillers were known to have an excellent burn, while the South Carolina filler was markedly inferior in this respect and the Ohio filler intermediate in burning qualities. It will be seen that this sample of wrapper burned quite evenly when used with the imported Cuban and Texas fillers, while with the Ohio and especially the South Carolina fillers the burn was decidedly uneven.

On the other hand, the effect of using different types of wrapper on the evenness of burn of any one type of filler was less marked (see Pl. IV, A, B, and C; also Pl. III, D). The filler used in this experiment was the imported Cuban, while the wrappers were taken from four different types of Sumatra tobacco grown in Connecticut. Of these four types of wrappers, that shown in Plate III, group D, had the best burn and the one shown in Plate IV, group C, the poorest, although little difference could be seen between the two when smoked on the Cuban filler. The use of different binders did not cause any marked differences in the evenness of the burn, as is shown in Plate IV, groups C and D. The cigars used in this experiment were all made from the same wrapper and the same filler, while in group D a sample of Connecticut Broadleaf tobacco was used as the binder, and in group C the binder was taken from the same leaf as the wrapper.

Another important factor in determining the evenness of the burn is the proper balancing of the component parts of the cigar. It was found, for example, that a very light[a] wrapper will not give good results on a heavy filler, even though both of these may in themselves possess a good burn. It will readily be seen that a very thin wrapper which burns readily and very rapidly will, when placed on a heavy, slow-burning filler, tend to burn in advance of the latter, and the effect will generally be an uneven burn. The same result is obtained when any cigar is smoked very rapidly, for the reason that the oxygen of the air has freer access to the outer edges of the burning zone and under the added stimulus it rarely happens that a cigar will burn evenly.

As regards the character of the ash, the wrapper and the binder are relatively of much more significance. It was found, it is true, that some fillers give an ash lacking in compactness and liable to split asunder, but the tendency to flake seems to be controlled almost entirely

[a] The terms *light* and *heavy* as used in this connection refer to the body or thickness of the leaf, which largely controls the rapidity of the burn.



VARIATION IN BURN OF FILLERS DUE TO DIFFERENT WRAPPERS AND BINDERS: A, B, C, THREE DIFFERENT TYPES OF CONNECTICUT-GROWN SUMATRA WRAPPER ON SAME SAMPLE OF CUBAN FILLER, BINDER BEING SAME AS WRAPPER IN EACH CASE; D, SAME WRAPPER AND FILLER AS C, BUT CONNECTICUT BROADLEAF USED AS BINDER.

by the character of the wrapper and binder. Furthermore, this lack of cohesion in the ash of a wrapper may be largely overcome by the use of a good binder. As to the color of the ash, a binder having a good burn will impart to the ash of the wrapper a lighter tint and a more uniform color. The general results of these experiments may be summarized as follows:

(1) In order to secure a good burn, due consideration should be given to the proper balancing of the components of the cigar; that is, a heavy filler should be wrapped with a comparatively heavy wrapper, while a light-bodied filler requires a light-bodied wrapper.

(2) Of the three components of the cigar, the filler exerts the strongest influence on the evenness of the burn.

(3) The influence of the wrapper and binder is shown most strongly on the character of the ash, and the binder very materially influences the ash of the wrapper in this respect.

TESTING THE CAPACITY FOR HOLDING FIRE AND THE EVENNESS OF THE BURN.

The factors of holding fire and of burning evenly are of prime importance in judging the burn of tobacco, and any sample found markedly deficient in these points may be rejected without applying any further test. As has been previously stated, the old method of measuring the fire-holding capacity is likely to lead to erroneous conclusions, while there has heretofore been no direct method of determining the evenness of the burn of wrapper leaf. In the process which we have used for testing wrapper tobacco with regard to these elements of the burn, the area of the leaf consumed, rather than the time elapsing before the glow is extinguished, is measured.

The form of the apparatus used in this method will be understood by reference to the accompanying illustration (fig. 3). The essential feature is the form on which the leaf is wrapped, consisting of a collapsible wooden tube, one end of which fits into a glass tube of the same diameter. This latter is in turn connected with a second glass tube through which is drawn a current of air. The best material for making the wooden form is well-seasoned cherry with a straight grain, but ash has also been found to answer the purpose very well. From the wood selected is made a cylinder 5 inches in length, $\frac{3}{4}$ inch in diameter at one end, and tapering slightly to the other end (see fig. 4). In the larger end of the cylinder a $\frac{3}{8}$-inch hole is bored to a depth of $3\frac{3}{4}$ inches, and the shell thus formed is separated into six equal segments by sawing to a depth of $3\frac{1}{2}$ inches. The smaller end is cut down for a distance of $1\frac{1}{2}$ inches, so as to fit snugly into the glass tube. The shoulder thus formed should correspond in depth to the thickness of the wall of the glass tube. Near the larger end of the form a groove (*c*) is cut, into which is fitted a rubber band. The plug (*d*) has a diameter such

that when inserted in the end of the form the latter is expanded to its original size. The receiver (*a*) for the form is made by drawing out

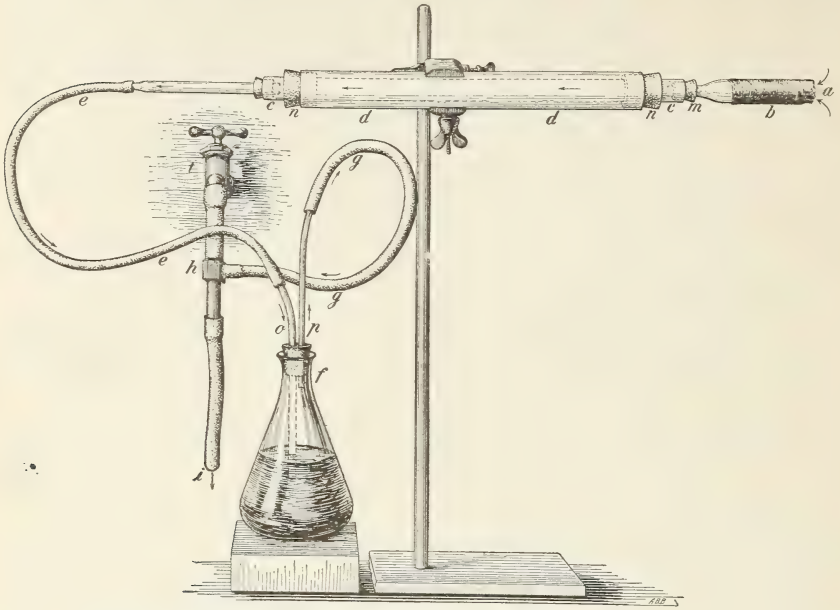


FIG. 3.—Apparatus for testing the burning quality of wrapper tobacco: *a*, entrance of air current; *b*, wrapper to be tested; *c*, *c*, glass tube to which the form bearing the wrapper is attached by means of the cork, *m*; *d*, *d*, large glass tube fitted with corks, *n*, *n*, through which passes *c*, *c*; *f*, flask containing water; *o*, small glass tube dipping beneath the surface of the water in *f*; *p*, short glass tube leading from *f*; *h*, pump by means of which the current of air is secured; *e*, *e* and *g*, *g*, rubber tubing connecting parts of apparatus; *t*, water tap; *i*, outflow of water.

one end of a short piece of thick-walled glass tubing. All of the above dimensions are based on tubing having an internal diameter of 14 mm. (9 16 inch) and an external diameter of 18 mm. (11 16 inch). The small end of the receiver is fitted with a soft cork (*b*), by means of which it is connected with the other portion of the apparatus.

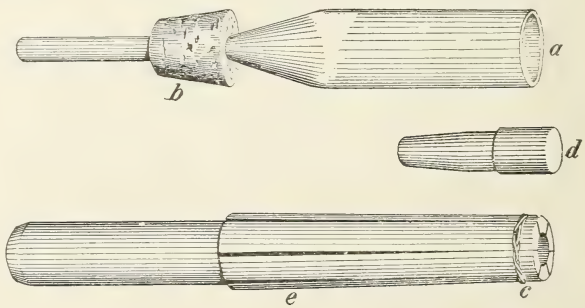


FIG. 4.—Construction of form on which leaf is wrapped for use in apparatus shown in figure 3: *a*, glass tube for receiving the form; *b*, cork by which receiver (*a*) is connected with remainder of apparatus shown in figure 3; *c*, rubber band for collapsing the form; *d*, plug for expanding the form; *e*, form on which leaf is wrapped.

From the leaf to be tested, which should be quite damp, the wrapper is cut into a form quite similar to that used for cigars, and the same rules are observed as regards the cutting of right-handed and left-handed wrappers, etc. Beginning at the outer end the wrapper is

rolled quite tightly, first on the form and then on the glass. At the beginning of the process of rolling, the extreme outer corner of the base of the wrapper is attached to the overlapping portion with a bit of cigar paste, and at the end of the operation the tip of the wrapper is attached to the receiver by the same means. A number of samples to be tested are thus wrapped on the forms and set aside until they have dried out properly. The plug in the end of the form is then withdrawn and the rubber band causes the walls of the latter to collapse, so that it can be easily withdrawn from the receiver. This leaves the sample of wrapper securely attached to the glass tube, and in exactly the same form it would have on a cigar. The tube carrying the sample to be tested is connected with the remainder of the apparatus, shown in figure 5, the construction of which will be understood without further explanation. The current of air is furnished by means of an ordinary filter pump, and its rate can be controlled with sufficient accuracy by measuring the flow of water through the pump. The end of the wrapper is ignited with a flat gas flame, and the evenness of the burn and the portion consumed before it ceases to glow are carefully noted. Our method of recording the results is to grade each sample on a scale of ten, both with reference to the evenness of the burn and the fire-holding capacity. Of course, standards in these tests are purely arbitrary, as the results are only intended to be comparative. Under the conditions laid down for the experiment, wrappers having markedly good burning qualities will burn up completely and evenly with only one lighting, and these are given a grade of 10.

For the purpose of comparing the results obtained by this method with those given by the cigar test with reference to the evenness of the burn, a number of leaves were selected from different types of wrapper tobacco. One half of each leaf was used for wrapping a cigar and the second half was wrapped on the form for testing, as has just been described. There was a decided lack of agreement in the results obtained by the two methods when only one type of filler was used in making the cigars. It was found that frequently a wrapper that graded only 5 or 6 on a scale of 10 in what may be called the "form test" would burn quite evenly on the cigar, whereas another wrapper grading as high as 9 in this test would show an uneven burn on the cigar. A good illustration of this point is found in a wrapper which was scored 10, 9, 10, respectively, in three experiments with the form test and gave a fire-holding capacity of 65 seconds by the old method of Nessler. On one type of filler this wrapper gave a very uneven burn, but when smoked on a lighter filler the burn was perfectly satisfactory. These results, then, seem to emphasize the fact that, although the final judgment as to the burning qualities of a wrapper which has shown up well in the preliminary tests must be based on the smoking

of the cigar, great care must be exercised to avoid the sources of error in this test which have been previously discussed. The test should not only be repeated with a single type of filler to avoid the effects of any possible unevenness or other imperfections in the manufacture of the cigars, but at least two different types of filler should be used, one of these being heavy and the other light in the sense in which these terms are used here.

TESTING THE BURN OF CIGAR-FILLER TOBACCO.

Testing the burn of a filler is a much simpler problem than is the case with a wrapper. The principal elements of the burn are the evenness and the capacity for holding fire, and the character of the ash is unimportant, except that it should be compact. The evenness of the burn and the fire-holding capacity are best determined by using the cigar test. In the case of filler tobacco the capacity for holding fire thus refers simply to the length of time the cigar will continue to burn after being lighted without being puffed by the smoker. The effects of the binder and wrapper on the burn may be avoided by making the entire cigar from the filler leaf to be tested. Another decided advantage in making the whole cigar from the same tobacco is that the aroma, which is so important in the filler, can also be tested at the same time. In determining the fire-holding capacity it is only necessary to light the cigar and test it at gradually increasing intervals of time to find whether it has ceased to burn. It is, however, desirable to test the fire-holding capacity and the evenness of the burn on separate cigars if sufficient material is at hand for this purpose.

B. P. I.—229.

V.—THE DRUG KNOWN AS PINKROOT.

By W. W. Stockberger, *Expert, Drug-Plant Investigations.*

INTRODUCTION.

The drug known as pinkroot is derived from the underground portions of *Spigelia marilandica* L. (Pl. V), an American herb now found growing most abundantly in the Southern States and occurring locally in the Mississippi Valley and eastward. It came into use in America as a vermifuge about 1723 and because of its valuable properties soon came to occupy an important place in materia medica. Unfortunately, however, conflicting reports on its physiological effects in time established for pinkroot a reputation for uncertain action, and within the last fifty years the use of this drug, once regarded as highly reliable and valuable, has greatly decreased. Since it has seldom been held at high prices the cost has not operated to drive it out of the markets.

The cause of the apparent loss of high efficiency formerly claimed for pinkroot has engaged the attention of students of crude drugs for many years. The demonstration by Dr. R. H. True[a] that an unsuspected substitute had crept into the markets and to a considerable degree replaced the true article has explained in large measure the unfavorable commercial and medical status of pinkroot. The results here outlined of a detailed study of pinkroot and its more important adulterants may serve to aid collectors in discerning the real pinkroot and to assist drug experts in distinguishing the plant from its sophistications in its commercial form.

TRADE VARIETIES OF PINKROOT.

The complex nature of the material put upon the market as pinkroot has long been known to the drug trade, and although the real nature of the spurious article was not understood, its presence was recognized, and various sorts of pinkroot came to be distinguished by definite trade names—e. g., true pinkroot, genuine pinkroot, southern pinkroot, Georgia pinkroot, East Tennessee pinkroot, western pinkroot, and true fiber pinkroot. The visible differences by which these trade varieties are segregated may be utilized in distinguishing

[a] Pharmaceutical Review, 21: 364, 1903.

the true from the false pinkroot, as it is now definitely known that certain of the trade varieties are wholly composed of worthless substitutes.

In many cases careless or unscrupulous collectors and dealers have not regarded the distinguishing features of the various sorts of pinkroot, or have been ignorant of them, with the result that very general confusion exists as to the character of the real drug and its adulterants. The authors of some prominent publications on crude drugs have evidently based their observations on trade varieties of pinkroot, as they have illustrated and described one of the most important adulterants as true pinkroot.

IDENTITY OF CHIEF SUBSTITUTES.

East Tennessee pinkroot (*Ruellia ciliosa* Pursh), a member of the plant family Acanthaceae, is the most important adulterant. Observations on the plant structures present in commercial samples of pinkroot convinced Dr. R. H. True, in 1900, that the chief part of this crude drug consisted of a substitute instead of Spigelia and that this substitute was a species of Ruellia. In trying to get plants of pinkroot for cultivation, Doctor True, in 1903, purchased several hundred roots from a dealer in eastern Tennessee. These roots were set out in the testing gardens of the Bureau of Plant Industry at Washington, D. C., and the plants kept under close observation. On developing they were found to differ markedly from Spigelia, and upon flowering were identified as *Ruellia ciliosa* (Pl. VI), a plant which had never appeared in the list of suspected adulterants prior to this time.

The examination of the microscopic structure of this plant recalled at once the figures in some text-books purporting to represent Spigelia and those illustrating an article by Greenish in the Pharmaceutical Journal, 1891, on the structure of *Phlox carolina*, a plant which had long been regarded as an extensive substitute for pinkroot. It was evident that a double confusion existed with regard to Ruellia. On the one hand it was so widely mistaken for Spigelia that its peculiar structures have been regarded as diagnostic of pinkroot, and on the other it was recognized as a substitute, but wrongly regarded as Phlox, a plant lacking many of the striking characteristics of Ruellia.

In order to satisfactorily determine the relation of these substitutions to the true pinkroot, observations have for three years been made upon plants of Spigelia, Ruellia, and Phlox under cultivation at Washington, D. C., and fresh material secured from them has been used in making a comparative study of their structure. The results of this study do not support the view that Phlox is an adulterant of pinkroot, and, moreover, several samples of a substitution supposed to be Phlox have proved, upon examination, to be composed entirely of Ruellia. It is only through long and familiar observation in the

PINKROOT (SPIGELIA MARILANDICA L.).

living condition of all species here concerned that it has been practicable to uncover fully the true relations involved in the drug called pinkroot.

MINOR ADULTERANTS.

Aside from Ruellia, the adulterants of Spigelia may be regarded as impurities, due in the main either to the carelessness of the collector in not sorting out the roots with which the plant was associated in its growth, or perhaps to a lack of familiarity with the plant on the part of young or inexperienced collectors. With Spigelia other roots sometimes occur which have a greater market value than the true pinkroot, and therefore can not be regarded as intentional adulterants. The worthless roots frequently present, however, may have been introduced by the collector with full knowledge that a fraud was being perpetrated. In commercial samples of pinkroot, among other impurities have been observed roots of golden seal (*Hydrastis canadensis* L.), serpentaria (*Aristolochia serpentaria* L.), soapwort (*Saponaria officinalis* L.), wild yam (*Dioscorea villosa* L.), and stoneroot (*Collinsonia canadensis* L.).

METHODS OF DISTINGUISHING PINKROOT FROM ITS SUBSTITUTES.

Once familiar with the true pinkroot it is hard to see how any drug collector could confuse it with the plants so frequently substituted in its place. Spigelia and Ruellia grow over large areas, largely overlapping and in much the same habitat, and have on the whole a certain general resemblance; but they should be readily distinguished by observing any one of their several striking characteristics.

In Ruellia (Pl. VI) the flowers are borne scatteringly along the stem in the axils of the leaves; in Spigelia (Pl. V) they are aggregated at the top of the plant in a one-sided spike. In Ruellia the pale magenta-colored corolla forms a slender tube below, expanded upward into a broad, flaring limb. The anthers and style are not protruded. In Spigelia the corolla forms a rather broad tube, narrowest at the throat, prolonged upward into spreading, narrow, triangular portions. The exterior is brilliant cardinal in color, bright yellow on the inside; the style and anthers are exserted. The leaves of Ruellia are bright green, usually short-petioled or sessile, frequently more or less hairy. In Spigelia they are dark green, glossy, and sessile.

In the crude drug the forms are separated by less evident gross characters. Ruellia, however, has a coarser, harsher root system than Spigelia, and the roots show a tendency to lose the cortical tissues, leaving the naked, woody cylinder exposed. The roots of Spigelia are delicate, fibrous, and usually very numerous. When dry they break and crumble very readily.

The differences in structure between Spigelia and its substitutions as seen under the microscope are usually very marked. This is especially true of Ruellia, the only important adulterant found with Spigelia, since the other plant parts sometimes present are usually recognized by their gross characters. A comparison of figures 5 and 6 will show the most important differences in minute structure. The numerous cystoliths present in Ruellia as a very conspicuous feature are wholly lacking in Spigelia and *Phlox ovata*. The large sclereids of the root of Ruellia are not found in the other two plants, and starch, which is present in Spigelia, is absent in both Ruellia and Phlox.

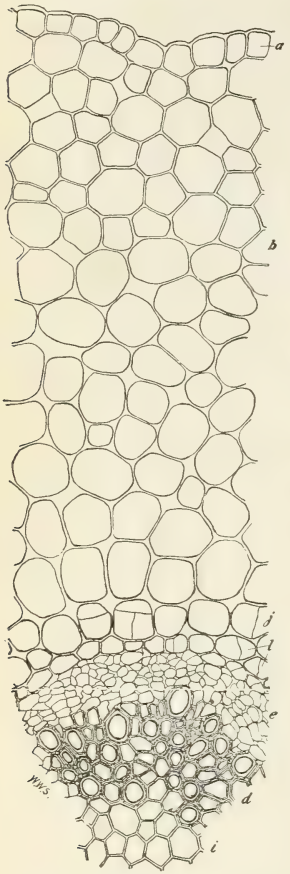


FIG. 5. Cross section of the root of *Spigelia marilandica* L.: *a*, epidermis; *b*, cortex; *d*, xylem; *e*, cambium; *i*, pith; *j*, endodermis; *l*, pericycle. × 180.

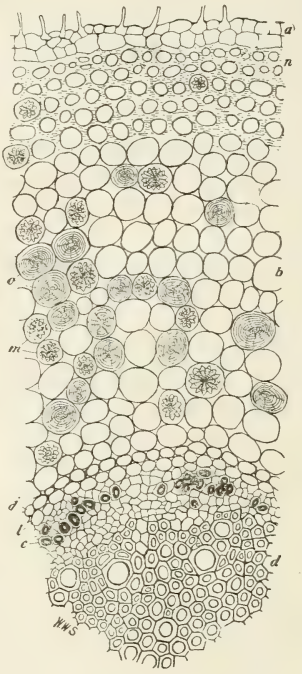


FIG. 6. Cross section of the root of *Ruellia ciliosa* Pursh: *a*, epidermis; *b*, cortex; *c*, bast fibers; *d*, xylem; *j*, endodermis; *l*, pericycle; *m*, cystoliths; *n*, collenchyma; *o*, sclereids. × 150.

In the powdered form Ruellia always reveals its presence by the numerous stone cells and cystoliths, which usually remain intact even in finely powdered material. Powdered samples of the underground portions of the Phlox at hand gave no reaction for starch. The fineness of the starch grain of Spigelia and its lack of striking characters render uncertain its identification among many other plant starches which might be readily introduced in the powdered drug. The starch grains of Spigelia measure about 4 μ, and in powdered pinkroot are associated with parenchyma cells and long light-colored sclerenchyma fibers. The absence of starch from a powder supposedly made of pinkroot suggests at once that the material is not Spigelia. On the other hand, the presence of starch, while indicative of Spigelia, is by no means conclusive proof of its presence.

EAST TENNESSEE PINKROOT (RUELLIA CILIOSA PURSH).

B. P. I.—230.

VI.—ORCHARD GRASS.[a]

By R. A. Oakley, *Assistant Agriculturist, Farm Management Investigations.*

INTRODUCTION.

Orchard grass (*Dactylis glomerata* L.) is a well-known standard grass which is grown to some extent in every State in the Union and quite commonly in the region east of the Mississippi River and north of the northern portions of Alabama and Georgia. It attains most importance, however, in Kentucky, southern Indiana, Tennessee, North Carolina, Virginia, West Virginia, and Maryland, and seems quite thoroughly adapted to a variety of soils in these States.

It may be said that the general opinion of farmers in regard to the value of orchard grass either for hay or pasture is quite unfavorable. This unfavorable opinion, which is due somewhat to prejudice, exists to a greater extent in the timothy region than elsewhere, and as the limits of this region are reached and crossed orchard grass is much more highly regarded. The objectionable features of the grass are in general its bunchy habit, coarseness, and the unpalatability of its hay unless cut at the proper state of maturity. These objectionable features are not alone the cause of its unpopularity or the reason why it is not grown more generally. There is no doubt that orchard grass could be grown very successfully throughout the greater portion of the timothy region, but as the demand for any hay except timothy is very limited farmers see little inducement for them to raise it. On

[a] In connection with the general plan of the Farm Management work it is contemplated to take up the study of various seed crops. Much interest is now being manifested in better seeds for the farmer. This is especially true of forage-crop seeds, including both grasses and clovers. Mr. Oakley's paper, which is contributed from the Office of Farm Management, conducted under the direction of Prof. W. J. Spillman, is a valuable contribution to the methods followed in growing orchard grass for hay, for pasture, and for seed. Special attention is called to the fact that orchard grass seed as usually grown is for all practical purposes pure. The investigations of this Bureau have shown that considerable quantities of the seed of this grass found in the market contain seed of other and less desirable, cheaper grasses. That the seeds of these cheaper forms have been added for the purpose of adulteration seems evident from the fact that the grass bearing them are not found in orchard grass fields to any extent worth mentioning.—B. T. Galloway, *Chief of the Bureau of Plant Industry.*

account of its maturing well with a number of other very valuable grasses and clovers its popularity may in time increase as the advantages of such mixtures become more generally appreciated.

Orchard grass is exceedingly variable and offers a large field for selection and breeding. Its variable characters of most importance are its coarseness, bunchiness, and time of maturing. By consistent selection with special reference to the first two characters valuable strains may in time be developed which will not possess the objectionable features of the common orchard grass now being grown.

METHODS OF CULTURE.

SEEDING.

While there are some methods that are generally employed in the culture of orchard grass, still there is a great difference of opinion even among the most successful growers in any one locality as to the best practices. In the seed-producing section of Kentucky and Indiana it is the common custom to sow the grass in February on fall wheat at the rate of from three pecks to one bushel to the acre. Since the crop in this section is almost entirely harvested for seed, it is not considered desirable to sow more than a bushel. In years past as much as two bushels to the acre were sown, but it is now the general opinion that one bushel is sufficient, and even less is often used. A bushel of orchard grass seed weighs 14 pounds. Orchard grass to give large yields of seed should be reasonably thin, as it produces more abundantly when in this condition. It is usually sown broadcast, as it does not feed out well through a press drill, either by hand or with a wheelbarrow or other type of seeder, and is covered very shallow. Good results are often obtained by not covering the seed, and it is quite a common opinion that too deep covering is the cause of many of the failures to secure a stand.

A method of seeding which is often followed in the section mentioned is to scatter the orchard grass straw from which the seed has been thrashed on ground that has been sown to wheat. This is usually done in February. The straw acts as a mulch in this case and the seed needs no covering. It is very essential that it be scattered evenly and very thin; otherwise the stand will be too thick and unsatisfactory. The greatest objection to this practice is that unless the straw is very clean the meadow is sure to be weedy, and some are of the opinion that since the seed that is left in the straw or blown over with it is mostly of poor quality a field of inferior and unequally maturing grass will be the result.

Orchard grass may be sown successfully after corn by splitting the rows with a disk harrow as soon as the crop is removed. This may be done any time during the month of October, and in February or as

soon as the weather is favorable the grass may be sown with a broadcast seeder at the ordinary rate, and the ground being uneven at this time the freezing and thawing which follow will cover the seed sufficiently. Disking seems to give much better results than plowing, since the ground if plowed will not have time to thoroughly settle before the time of seeding. Rolling would doubtless be beneficial after seeding in this manner.

In western Virginia and in Tennessee orchard grass is commonly sown the latter part of September or the first of October with wheat on the ground at the rate of a bushel and a half to two bushels to the acre, the wheat being drilled in at the rate of three to five pecks to the acre and the grass sown either broadcast by hand, with a broadcast seeder, or an attachment to the drill, and covered as shallow as possible.

Orchard grass is often sown with oats, usually in March, on ground that has been previously in wheat and which has been plowed the preceding autumn. A half seeding of oats is usually sown in this case, and gives as a result only a fair crop. The grass, however, makes more pasture the first season as a rule than when sown with a full seeding of either wheat or oats. Early fall seeding with winter oats in sections where the latter can be grown may be depended upon to give good results, but on account of the Hessian fly it is not possible to sow it with wheat much before October.

A crop of hay is not expected the first season, whether the grass is sown in the fall or spring, either alone or with a nurse crop. If sown alone a light cutting may be secured, provided the conditions are favorable, in the latter part of August or September; but in general the grass is pastured and not cut except at the time when the grain which is sown with it is harvested. The following season it makes a crop of either hay or seed, as is desired. There may be some advantage in sowing the grass alone for the extra quantity of forage produced the first year, but whether this and any other advantages that may come from seeding in this way will compensate for the profit accruing from the nurse crop is an undecided question.

MIXTURES WITH RED CLOVER.

Throughout almost the entire region where orchard grass is grown it is quite a common practice to sow red clover with it. This practice is a good one, not only for the value of the red clover in maintaining the soil fertility, but also for the fact that its presence greatly improves the orchard grass either for hay or pasture. In the seed-producing sections red clover is a menace to the seed crop, especially the first and second years. As it is impossible to cut the orchard grass above the clover, the leaves and heads get mixed in with the seed and are difficult to separate from it. Although the presence of the leaves in the

orchard grass seed materially decreases its commercial value, the advantage of having the clover in the field more than compensates for this.

In sections where the grass is grown for hay and pasture, red clover is sown at the rate of one bushel to 5 or 7 acres, usually as early in the spring as the weather will permit. It is, however, sometimes sown in the autumn at the same time as the orchard grass, but the seed of the two are not mixed, as they do not feed evenly through the drill or seeder. In cases where clover is sown in the spring on orchard grass that has been sown the fall before, it is either covered lightly by means of a drag harrow or left uncovered. Less clover is sown to the acre in seed-producing sections than where the grass is intended for hay or pasture, the customary quantity being one bushel to 8 or 10 acres. In these sections the first crop is frequently cut for hay on account of its containing so much clover. The second crop usually contains very much less and is cut for seed, as are also the following crops, since the clover at the end of two years usually disappears. Pasturing the field appears to materially increase the longevity of the clover, and it is not uncommon to see meadows that are 6 or 7 years old containing almost as much clover as they did the first year.

Much trouble is now being experienced in securing a catch of red clover throughout the greater part of the region where it is grown. As a result alsike is being substituted in some sections, and where it has been tried thoroughly it is giving good results. This difficulty in growing red clover will doubtless soon become a serious proposition, especially in seed-producing sections, and unless alsike or some similar leguminous crop can be grown it will be only a few years until more barnyard manure or commercial fertilizer will have to be used. At the present time there is very little commercial fertilizer applied to orchard grass, although it is the opinion of some of the more successful growers that an application of about 200 pounds of good fertilizer in the spring, just as the grass begins to grow, would yield profitable results.

MIXTURES WITH OTHER GRASSES.

On account of the bunchy tendency of orchard grass it is often desirable to mix it with other grasses for hay or pasture, and while this has not been practiced as yet to any great extent the results obtained from such mixtures are very promising. Aside from affecting the palatability of the grass, the mixtures have a tendency to increase the yield. Orchard grass matures well with tall meadow oatgrass and meadow fescue, and in some localities in Tennessee a mixture of it with the latter is attracting considerable attention, especially for pasture. Doubtless in time orchard grass will be more generally grown for hay and pasture in mixtures with these or other grasses.

LIFE OF MEADOWS.

Orchard grass is a more hardy and permanent grass than timothy, and as a result remains productive in a meadow under most conditions much longer. In the principal sections where it is grown the average life of a meadow is from five to seven years, although it is a question whether it might not be broken up profitably at the end of four years. Throughout almost its entire region Kentucky bluegrass is its natural enemy and works in around the bunches almost to its ultimate exclusion. Redtop and Canada bluegrass also are present in many sections with the Kentucky bluegrass, and at the end of five or six years these three grasses are greatly in the majority. Pasturing seems to facilitate the growth of the bluegrass, inasmuch as it has a tendency to cause the orchard grass to become more bunchy, and it is also a means of spreading the bluegrass seed. During the last year of its existence it is customary to pasture the orchard grass field, and late in the autumn or early in the winter it is broken up and is planted to corn the following spring. It is well to have the field broken up as early as possible so as to give the sod time to rot sufficiently before planting the corn.

USES AND VALUE.

HAY.

According to chemical analysis orchard grass hay should be equal, if not superior, to timothy, but in real practice it does not seem to be able to successfully compete with the latter. In large cities there is practically no demand for any hay except timothy, and the demand for orchard grass hay is only local and very limited. In the timothy region orchard grass is looked upon very unfavorably, but where timothy can not be grown so successfully its hay is used to a greater extent and is considered of very good quality.

As previously stated, orchard grass should be sown thicker when desired for hay than for seed, 2 bushels of good seed to the acre being usually required, for unless thick it becomes coarse and woody. Its value as hay is increased by the addition of red clover or alsike, and where it has been sown with other grasses, such as tall meadow oat-grass or meadow fescue, its quality seems to be improved by such mixtures. The state of maturity at which the grass makes the best hay is when it is just in bloom. Not only does the quality seem to be better at this time, but the yield is also at the maximum.

In some sections it is considered a good hay for horses, but it is of more value for cattle, and especially for fattening them for the market. As a feed for sheep it is of only fair quality. The value of the hay depends not only on the state of maturity at which it is cut, but also on the bunchiness and coarseness of the grass. These characteristics

are influenced largely by the method of culture, and it is often for the reason that farmers do not thoroughly understand growing it that they condemn it as a hay grass. Seeding evenly with the proper quantity of seed and careful pasturing are important factors in securing a good meadow.

PASTURE.

For pasture, orchard grass gives best results in mixtures with other grasses and clovers, and is of special importance from the fact that it can be grazed early and late in the season. It is quite a valuable grass if for no other reason than this. It also stands grazing fairly well. It must, however, be closely pastured; otherwise it will become too coarse and woody, and stock will not eat it. Stock do not relish the mature grass, and invariably lose flesh when turned on a field in this condition. To secure best results from pastures, they should be mowed some time during June to keep down the weeds, and again later as needed. In this way they are kept clean and more productive. Bluegrass and white clover are usually very prominent in most orchard grass pastures and are valuable additions, as they grow between the bunches of the orchard grass, thus increasing the yield. The white clover is also of value in maintaining soil fertility.

SEED.

Orchard grass seed is produced to some extent throughout the entire region in which it is grown. There is quite a quantity raised in western Virginia, but the greatest seed-producing area is in the vicinity of Louisville, including Jefferson, Oldham, and Shelby counties, Ky., Clark County, Ind., and some of the counties adjoining those in both States mentioned. Just why there is more seed produced in this section than elsewhere is not definitely known. Some are of the opinion that it is because orchard grass seeds more readily there, which may be true. However, the cultural methods employed by farmers in this section may have something to do with the success attained in raising it. In Oldham County, Ky., the average production is about 55,000 bushels, which represents practically 5,000 acres, as the yield is about 10 to 12 bushels to the acre. The growing of seed in the section referred to is a profitable industry, and there are many farmers who engage in it quite extensively with uniform success. It is said to be a more profitable crop than wheat, and when the harvest of the two conflict orchard grass is given the most attention. The average price of seed for the last ten years has been about $1.25 a bushel. The seed alone does not represent the entire return from the field, for after it is harvested the meadows afford hay or pasture, or both, from which a considerable profit accrues. Orchard grass seed is the controlling crop in this section, and the cropping system is planned to accommodate it.

FIG. 1.—HARVESTING ORCHARD GRASS FOR SEED.

FIG. 2.—METHOD OF SHOCKING ORCHARD GRASS; SHOCKS SHOWING BANDS AT TOP.

HARVESTING THE SEED CROP.

The methods used in harvesting orchard grass seed are practically the same throughout the whole country. In general, harvest begins about June 15 and lasts about ten days, though when there is a large acreage it is often necessary to begin earlier than this, in order to finish before the seed becomes too ripe. An inferior quality results from cutting the seed before it is sufficiently mature, and this seed is quite readily detected by its light-green color. When properly matured the seed is straw colored, and not at all green. A common test to determine whether the seed is at the proper stage for cutting is to beat the heads in the palm of the hand, and if quite a quantity shatters off it is considered ready to cut. To one unfamiliar with the crop it would seem that the waste from shattering would be great.

Orchard grass is harvested with an ordinary grain binder (Pl. VII, fig. 1), making as small bundles as possible, in order that they may cure readily. The bundles are placed usually three in a shock and the shocks tied at the top with two bands of straw, one about 8 inches below the other (Pl. VII, fig. 2). They are bound in this way so as to make them more stable and to prevent the seed from shattering. The shocks are made small to facilitate handling at the time of thrashing, and so that they may be easily tied at the top with the straw bands. They are left standing from two to four weeks, or until they have had time to cure thoroughly, and are thrashed without stacking. On an average it takes about 5 pounds of twine for 100 bushels of seed. The crop is of such importance that the fence corners and other places that can not be reached with the binder are cut with the cradle and bound into bundles by hand.

When the grass is sufficiently tall it is cut from 12 to 14 inches high, to avoid the low-growing weeds, such as plantain and sorrel, and also clover and bluegrass. Another advantage in high cutting is that it leaves more of the undergrowth to be utilized later for hay or pasture.

THRASHING.

The common grain separator is used for thrashing with the ordinary cylinder and concaves, but with special riddles and with nearly all the wind shut off to prevent too much of the seed from being blown over. In hauling the shocks to the machine, racks with tight beds or with tarpaulins spread over the bottom are used to catch the seed that shatters off, which is usually considerable. This is always heavy seed, and is worth saving. Since the shocks are small, a whole one may be thrown on the rack at one forkful without breaking the bands. This reduces shattering to a minimum. Unless the grass is very weedy the thrashing machine cleans the seed sufficiently for the market, but most of the larger growers have hand fanning mills, which are used when necessary. Seeds like those of redtop are easily blown out, but

it is harder to dispose of the bluegrass and some of the weed seeds, such as plantain and whitetop. From the machine the seed is put into 8-bushel bags for shipping. Thrashing costs on an average 8 cents per bushel, with the customary crew furnished.

HANDLING THE AFTERGROWTH.

In cases where orchard grass is cut for seed there is a great difference of opinion as to how the aftergrowth should be handled. It is generally considered that pasturing is not in the least detrimental but is even beneficial. Whether the aftergrowth should be cut for hay is an undecided question. It is a common practice, however, to cut it, due to the fact that it is depended upon largely for hay, since timothy and other hay grasses are not grown to any great extent. After the grass is cut for seed, especially when it is cut sufficiently high, there is always considerable green undergrowth. This continues to grow, and during the latter part of August or about the first of September is at the proper stage to cut for hay. If there is clover present in the aftergrowth it makes a very fair quality of hay and yields from one-half to one ton to the acre. The quality of this hay is not so good as that of the hay made from the first cutting.

While it is a general practice to cut the aftermath during the latter part of August or September, there are some who prefer to cut it as soon as the shocks are removed from the field, as it is believed to be better for the following seed crop if it is cut then. It is the opinion of some that the aftermath should be cut in any event, and consequently if it is not desired for hay it is cut and left on the ground. Others are of the opinion that if it is cut at all it materially injures the next year's crop, especially so if used for hay, as the two cuttings remove a large amount of plant food from the soil without much return.

Judicious pasturing, to say the least, is not detrimental to the field, and in all probability is more or less beneficial. The aftergrowth, which comes on after the seed crop is removed, furnishes grazing until it is covered with snow, and in the more southern sections where the grass is grown lasts nearly the entire winter. Sheep can be very profitably pastured on this aftergrowth, and in many cases almost as much money is made from the pasture that it affords the sheep as from the seed crop, on account of the length of time which it will furnish grazing. At present prices sheep are equally as profitable as cattle, if not more so, and can be pastured on orchard grass to much better advantage.

VALUE OF THE STRAW.

There is much difference of opinion regarding the value as a feed for stock of orchard grass straw from which the seed has been thrashed. Some state that it is of almost as much value as the hay, but in general it is thought to be about equal to wheat straw. Its value depends

largely on three factors: The state of maturity at the time of cutting; the amount of aftergrowth, including red clover, contained in it, and the success with which it is cured. If the grass is cut before the seed is sufficiently mature to harvest, the straw will be of more value for feed than when it is cut at the proper stage. The undergrowth which is present probably furnishes as much feed as the straw itself, if not more, especially when it contains clover.

It is a common practice to cut the grass as high as possible to avoid the weeds and clover, but if it is short there is necessarily a great deal of undergrowth cut with it. If the grass has not received too much rain while in the shock and is stacked properly or put into a barn or shed at the time of thrashing, the straw will be of much more value than if carelessly handled. In general, there is little attention paid to the stacking of the straw, and it is commonly left in piles just as they are made by the machine. When utilized for forage it is fed to horses or cattle, usually the latter, but is of very little value for sheep. Aside from its value as a feed, straw may be used for seeding meadows, as previously described. It should never be used for this purpose, however, unless thoroughly free from weeds.

WEEDS IN ORCHARD GRASS SEED FIELDS.

The weeds which are most troublesome in orchard grass fields, especially in sections where seed is produced, are whitetop (*Erigeron annuus*), red sorrel (*Rumex acetosella*), oxeye daisy (*Chrysanthemum leucanthemum*), milfoil (*Achillea millefolium*), and the plantains (*Plantago lanceolata* and *P. aristata*). Most growers pay much attention to keeping these weeds out of their fields and go to considerable expense for labor to mow them or cut them out with a hoe just before harvest. A method which is now quite commonly used and which is most effective and practicable is to pasture the fields with sheep. This is an excellent practice and it is comparatively easy to distinguish at harvest time between fields that have been pastured in this way and those that have not by the absence of weeds in the former. Such good results have been obtained by pasturing sheep on the grass to keep down the weeds that farmers are raising more sheep than formerly and are growing cleaner seed. It is a common practice to turn the sheep on in the spring as soon as the grass begins to grow and allow them to remain until the early part of May. As the grass advances toward maturity the sheep eat very little of it, but graze mostly upon the weeds and undergrowth, and especially on the whitetop, which is one of the worst weeds present, if not the worst. They do little damage to the field when it is dry, and in wet weather they are kept off, as they drag down too much of the grass. Although it is the custom to turn the sheep out of the fields in the early part of May, some of the most successful growers leave them in until nearly harvest time. It is

not uncommon to see sheep in fields that are ready to harvest. When it is possible to do so the fields should be pastured late, as this practice is more effective in keeping down the weeds, since it takes them but a short time to make sufficient growth to interfere with the cleaning of the seed. Cattle are sometimes pastured on fields that are intended for seed, but they tramp down too much of the grass and are not as satisfactory for this purpose as sheep.

OTHER GRASSES IN FIELDS INTENDED FOR SEED.

Much has recently been said regarding the presence of seeds of other grasses in orchard grass seed. Those which appear to be the most common are meadow fescue (*Festuca pratensis*) and the rye-grasses (*Lolium perenne* and *Lolium italicum*). The seed of these grasses is much heavier, but it resembles orchard grass seed to such an extent that its presence is not readily detected. Meadow fescue and rye-grass have very much the same appearance, but there is no difficulty in distinguishing them from orchard grass, as their seed habits and general habits of growth are different. If these grasses were present in considerable quantities in the orchard grass fields in Kentucky, Indiana, and western Virginia, where practically our entire supply of seed is produced, the presence of their seed in orchard grass seed could be readily accounted for. The orchard grass fields in these sections, however, are almost entirely free from other grasses, and only in a very few cases are there any others present, with the exception of some Kentucky bluegrass (*Poa pratensis*) and Canada bluegrass (*Poa compressa*) and a little cheat (*Bromus secalinus*) and redtop (*Agrostis alba*). Bluegrass and redtop, especially the former, come in naturally in the older fields, and cheat is present practically only the first year, due to its having been in the wheat which just preceded the grass crop. The quantity of meadow fescue and rye-grasses in these fields is insignificant, and there are only a very few cases where these grasses are present at all. The total percentage of other grasses in orchard grass throughout the whole seed-producing section is so small as to be hardly worthy of consideration, and statements made to the effect that the presence of their seed in orchard grass seed is due to the fact that they are grown with the orchard grass and can not be separated from it are entirely without foundation. Farmers in general are extremely careful to keep their orchard grass fields free from other grasses, for the reason that their seeds are readily detected by buyers and as a consequence the seed invariably sells at a lower price. It is a comparatively easy matter for seed growers to have pure seed for their own sowing, and there would be absolutely no advantage to them in growing meadow fescue, rye-grasses, and other grasses with orchard grass.

SUMMARY.

Orchard grass is of considerable value for early and late pasture, and in the southern part of the region where it is grown can be pastured nearly the entire year. When used for pasture, bluegrass and white clover are commonly grown with it.

Orchard grass hay is of value, especially when it contains red clover, and can be fed to horses successfully. It is a good forage for cattle that are being fattened for market.

When grown for seed, orchard grass is a profitable crop, as it yields on an average 10 to 12 bushels to the acre and sells for $1.25 a bushel. Aside from securing a crop of seed, the aftergrowth may be either pastured or cut for hay. This aftergrowth makes a very fair quality of hay, and when cut during the latter part of August or September gives a yield of from one-half to one ton to the acre.

Although not previously stated, orchard grass is quite valuable for binding soils, and on rough land that washes badly it can be used for this purpose effectively.

Orchard grass may be seeded either in the autumn or spring with about equally good results. Spring seeding, however, seems to be the most common practice. In most cases it is sown broadcast on fall wheat on fields that have been in wheat the previous year. One bushel of seed is a sufficient quantity when the grass is to be grown for seed. When grown for hay or pasture, more than this should be used. A good catch may be obtained by scattering the straw evenly and thinly on fall wheat in early spring.

Red clover can be profitably sown with orchard grass at the rate of 1 bushel to 5 or 7 acres. Mixtures of orchard grass with other grasses, especially with tall meadow oat-grass and meadow fescue, are giving good results for hay and pasture in places where they are being tried.

The average life of an orchard grass meadow is from five to seven years, after which it is plowed up, usually late in the fall, and put into corn.

Orchard grass is harvested for seed from about June 15 to June 25. It is cut with an ordinary grain binder and bound into small bundles, requiring about 5 pounds of twine to 100 bushels of seed. The bundles are put three in a shock and bound at the top with a band of grass to make them more stable and to prevent the seed from shattering. Thrashing is done from the shock after the grass has stood in the field from two to four weeks, with an ordinary separator, using special riddles.

Sheep are pastured on orchard grass in the spring to keep down the weeds. They are sometimes allowed to remain in the field until nearly time to harvest. This practice is very effective in keeping clean the fields that are grown for seed.

The percentage of meadow fescue, rye-grasses, and other grasses in orchard grass fields that are grown for seed is so small as not to be worthy of consideration.

VII.—THE EFFECT OF COPPER UPON WATER BACTERIA.[a]

By Karl F. Kellerman, *Physiologist in Charge of Soil Bacteriology and Water Purification Investigations,* and T. D. Beckwith, *Scientific Assistant.*

INTRODUCTION.

Of the many methods of chemical treatment for water purification, the copper method has recently received the most attention. The use of this metal, or its salts, was urged primarily[b] for the eradication or control of polluting algæ, though popular attention has been directed more particularly to the various results obtained in destroying the typhoid bacillus.

The discrepancies between laboratory results of some of the investigators of this phase of the subject have deterred many engineers and sanitarians from conducting important tests of the value of copper under emergency or epidemic conditions. These discrepancies are perhaps in some cases due to reasons which are taken up later in this bulletin, and in some cases to the lack of comprehension of the essential difference between treatment of drinking water which contains relatively little albuminoid matter[c] and treatment of bouillons or emulsions.[d]

[a] The copper method for controlling algal pollution can no longer be considered in an experimental stage. The accumulation of results of treatments constantly made under the direct supervision of officials of the Department of Agriculture and the numerous results reported to this Department by independent experimenters render further discussion of this question superfluous. The caution should be reiterated, however, that no rule for determining the amount of copper sulfate to be added can be given, and each body of water must be treated in the light of its special conditions. This caution is eminently applicable to copper treatment of a water supply for bactericidal purposes, and, as Mr. Kellerman and Mr. Beckwith have shown in their recent work, the problem of using dilute solutions of copper for destroying *Bacillus coli* and *Bacillus typhi* in water is a rather complicated one. Pertinent conditions must be understood thoroughly before an application of copper can be safely advised, and whether an emergency treatment of an unfiltered and contaminated water, or a continuous treatment of a filtered water supply, or a treatment of a sewage effluent is contemplated the work should be supervised by an expert.—A. F. Woods, *Pathologist and Physiologist, Acting Chief of Bureau.*

[b] Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[c] See also Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[d] A case in point is a paper by Dr. J. B. Thomas, Report on the Action of Various Substances on Pure Cultures of the Amœba Dysenteriæ. (Amer. Jour. Med. Sci., vol. 131, No. 1, Jan., 1906, p. 116.) "Uniform suspensions of the amœba were made by pouring 4 cc. of distilled sterile water over the surface of a 48-hour slant agar culture of the amœba and cholera spirillum, scraping off the surface growth and mixing with the matter by means of a platinum wire, and pouring the resultant emulsion into a sterile test tube; 4 cc. of the antiseptic solution (in double strength)

In spite of the fact that the field results of several experimenters[a] have apparently established the point that copper sulfate will not destroy ordinary water bacteria at concentrations fatal to the colon and typhoid bacilli, the somewhat fanciful objection has been suggested to chemical treatment of any kind, and to copper treatment in particular, that bacteria desirable for oxidation of organic matter and other beneficial changes in a water supply might be injured equally with the typhoid bacteria, and thus a treated water be more potentially dangerous than an untreated water known to contain typhoid organisms; in other words, that many bacteria which could be of decided benefit in a slightly polluted water supply might be eradicated by copper treatment and that in this way the fractional sterilization of a reservoir by copper might pave the way for a more dangerous contamination.

Investigations of these and other points have been undertaken, and it is believed that valuable data have been obtained, applicable to copper treatment for algæ, to emergency treatment for typhoid, and to copper treatment in connection with filtration. The copper treatment of sewage may be influenced by the same conditions that bear upon the effect of copper treatment of water. For the present, however, the investigations of Johnson,[b] at the Columbus, Ohio, sewage testing station may be considered sufficiently accurate for practical purposes.

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to be tested was then added to the 4 cc. emulsion of amœbæ, thus making a fairly uniform emulsion of 8 cc. of liquid to one 48-hour slant culture, the mixture containing a definite amount of the chemical to be tested. * * * It would appear from the above results that it would be disastrous to rely on the action of copper containers to purify water infected with amœbæ or cholera."

[a] Caird. Copper Sulphate Results. Paper read at meeting of American Water Works Association, Boston, Mass., July 10–14, 1906.

Jackson. Journal New England Water Works Association, vol. 19, 1905, pp. 563–568.

Hollis. Journal New England Water Works Association, vol. 19, 1905, pp. 571–572.

Stokes and Thomas. The Effect of Copper Sulphate upon the Bacteriological and Chemical Constituents of Large Bodies of Water. Public Health Papers and Reports, American Public Health Association, vol. 31, part 1, 1905, pp. 75–90.

[b] The Copper Treatment of Sewage Effluents. Report on Sewage Purification at Columbus, Ohio, 1905.

"Available data indicate that the removal by either process of applied pathogenic and nonpathogenic bacteria is in fairly direct proportion, generally speaking, although, of course, saprophytic bacteria may multiply within the tanks or filters, so as to obscure the true removal. Under some conditions it might be advantageous to employ a germicide, such as sulphate of copper, as a final treatment for sewage effluents of doubtful bacterial purity." (P. 471.)

"Independent of the question of complete sterilization as touched upon * * * it may be that there is a field of usefulness in some places for copper sulphate or other germicidal chemical in the treatment of coarse-grain filter effluents, in order to bring them from a bacterial or hygienic standpoint to a degree of purity strictly comparable with that of the effluent of ordinary intermittent sand filters." (P. 479.)

RESISTANCE OF VARIOUS BACTERIA.

Several species of bacteria occurring commonly in Potomac River water have been isolated, and comparative tests have been carried on with these bacteria and with the colon bacillus. An examination of the tables showing the exact results of these tests indicates clearly that no danger to most of the common saprophytic bacteria can be expected from using concentrations of copper sufficiently strong to destroy *Bacillus coli*. To allow as little variation as possible in the different tests all experiments were carried out in triple-distilled water. This will probably explain the sensitiveness of most of the bacteria to copper solutions, for, as Gildersleeve[a] has stated, "The resistance of bacteria washed in distilled water is lessened, due in all probability to the fact that some of the slimy substance which surrounds the bacterial cell and protects it to some extent from the action of detrimental agencies is removed." For purposes of comparison the table showing the action of copper sulfate on various bacteria in tap water, published by Gildersleeve,[b] is reproduced below.

Organism.	Dilution.	Average number to the cubic centimeter in control.	Percentage destroyed in hours specified.												
			1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	24.
B. typhosus	1: 250,000	110,000	100	....	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	100,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,000,000	140,000	78	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,500,000	120,000	50	85	99	100	....	....	....	....	....	....	....	....	....
	1:2,000,000	130,000	4	7	55	80	82	87	87	93	98	100	....	....	....
B. coli communis	1: 250,000	105,000	100	....	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	120,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1:1,000,000	99,000	80	99	100	....	....	....	....	....	....	....	....	....	....
	1:1,500,000	150,000	87	95	99	100	....	....	....	....	....	....	....	....	....
	1:2,000,000	110,000	8	10	14	27	50	85	90	92	93	93	95	98	100
B. dysenteriæ	1: 250,000	11,500	95	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	120,000	40	95	97	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	72,000	31	50	62	85	99	99+	100	....	....	....	....	....	....
	1:1,500,000	12,400	20	30	40	42	60	70	75	81	85	87	90	91	100
	1:2,000,000	13,600	20	20	25	30	30	45	60	62	62	68	71	75	98
B. cloacæ	1: 250,000	92,000	90	99	100	....	....	....	....	....	....	....	....	....	....
	1: 500,000	86,000	68	90	99	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	101,000	33	85	89	96	96	99	100	....	....	....	....	....	....
	1:1,500,000	89,000	5	10	30	40	70	85	95	98	98	99	100	....	....
	1:2,000,000	94,500	3	10	31	40	80	83	85	88	91	91	93	97	100
B. proteus vulgaris	1: 250,000	115,000	93	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	106,000	65	92	98	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	111,000	28	51	82	90	95	98	100	....	....	....	....	....	....
	1:1,500,000	99,000	10	18	27	42	69	90	93	98	99+	100	....	....	....
	1:2,000,000	118,000	0	12	22	36	51	65	78	80	85	88	92	95	100
B. prodigiosus	1: 250,000	102,000	95	99	99	99+	100	....	....	....	....	....	....	....	....
	1: 500,000	109,900	15	26	80	95	97	99	99+	100	....	....	....	....	....
	1:1,000,000	110,000	9	11	16	33	33	45	47	61	63	69	....	....	93
	1:1,500,000	107,000	4	12	17	30	38	46	46	48	51	52	60	61	92
	1:2,000,000	98,000	0	1	8	8	10	11	15	30	38	41	43	50	88
Staphylococcus pyogenes aureus	1: 250,000	92,000	99	100	....	....	....	....	....	....	....	....	....	....	....
	1: 500,000	88,000	50	70	90	100	....	....	....	....	....	....	....	....	....
	1:1,000,000	86,000	15	30	30	70	78	85	97	99	99	100	....	....	....
	1:1,500,000	100,000	8	22	28	96	58	69	85	93	95	99	100	....	....
	1:2,000,000	95,000	1	8	10	30	50	64	79	82	85	98	99	99+	100

[a] Studies on the Bactericidal Action of Copper on Organisms in Water. American Journal of Medical Science, vol. 129, 1905, p. 754.

[b] Op. cit., p. 759.

The figures in this table are rather surprising in connection with the statement that "very little difference was found between distilled and filtered water used in the laboratory." The similarity here of the action of copper in distilled and filtered tap water, contrasted with the marked difference in the action of distilled and filtered tap water in Washington, emphasizes a point previously mentioned,[a] that the water itself deserves as careful a study as do the organisms contained therein. This is suggested again in Phelps's[b] report showing that with either hard or turbid waters the germicidal efficiency of metallic copper is much lessened. Again, in view of the high toxicity that investigators have reported with copper in Philadelphia tap water, it seems necessary to assume that this water either is peculiarly favorable to maintaining the metal in a toxic state, or, what seems equally probable, for rendering the bacteria unusually sensitive.

The results of our own experiments are given in the following tables. All the experiments, unless otherwise indicated, were conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, portions of which had been treated previously with the desired amount of copper sulfate. All tubes were inoculated with a 2 mm. loop of the proper organism. The temperature during each experiment varied from 18° to 22° C.

TABLE I.—*Effect of copper sulfate upon Bacillus mycoides.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	105	90	590	310	70	250	90
6 hours	80	1	20	80	15	35	15
24 hours	185	10	130	110	40	20	50

TABLE II.—*Effect of copper sulfate upon Bacillus megatherium.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	70	55	45	30	90	15	50
2 hours	50	10	25	30	40	0	20
6 hours	40	15	20	10	40	1	15
24 hours	200	10	15	5	10	2	10

[a] Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agriculture, p. 12; and Kellerman, Journal New England Water Works Association, vol. 19, 1905, p. 536.

[b] Journal New England Water Works Association, vol. 19, 1905, pp. 537–539.

TABLE III.—*Effect of copper sulfate upon Bacillus mesentericus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	300	380	400	520	280	440	470
2 hours	280	170	310	440	260	340	360
6 hours	430	10	290	320	250	310	390
24 hours	1,350	0	0	530	3	4	30

TABLE IV.—*Effect of copper sulfate upon Bacillus mesentericus fuscus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	30	40	25	25	50	20	30
2 hours	0	5	50	10	3	0	1
6 hours	2	1	30	5	5	2	1
24 hours	70	110	70	45	0	10	80

TABLE V.—*Effect of copper sulfate upon Streptococcus pyogenes.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	180	875	875	300	250	90	180
2 hours	210	0	0	290	180	180	180
6 hours	140	0	0	280	230	240	160
24 hours	35	0	0	390	150	10	20

TABLE VI.—*Effect of copper sulfate upon Bacillus subtilis.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	310	80	20	130	85	100	30
2 hours	220	1	3	30	120	70	10
6 hours	330	1	1	25	85	50	2
24 hours	2,800	1	0	30	70	65	0

TABLE VII.—*Effect of copper sulfate upon Bacillus prodigiosus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	350	450	400	1,050	22,000	2,000	1,800
2 hours	750	65	180	1,700	1,350	2,050	1,550
6 hours	1,250	4	0	2	0	550	200
24 hours	925	0	0	0	0	0	0

TABLE VIII.—*Effect of copper sulfate upon Bacillus liquifaciens phosphorescens.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	16,500	3,400	2,400	16,000	4,500	10,400	13,000
2 hours	15,800	0	1	0	5	2,900	7,300
6 hours	23,000	0	0	0	0	640	7,000
24 hours	36,000	0	0	0	0	60	5,300

TABLE IX.—*Effect of copper sulfate upon pink yeast.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	310	550	100	110	100	220	108
2 hours	150	5	1	0	1	65	85
6 hours	70	0	0	0	1	3	50
24 hours	90	0	0	0	0	1	30

TABLE X.—*Effect of copper sulfate upon Bacillus coli.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.	2,000,000 parts of water.	3,000,000 parts of water.	4,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,650	2,300	3,100	2,700	2,250	1,800	1,770
2 hours	2,350	1,650	2,650	2,000	1,650	1,850	1,850
6 hours	2,300	0	0	95	210	850	1,070
24 hours	6,300	0	0	0	0	0	65

TABLE XI.—*Effect of copper sulfate upon sulfur yellow bacillus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,800	40	3,100	2,800	4,100	860	1,300
2 hours	2,000	0	0	0	280	1	2
6 hours	1,500	0	0	0	0	0	0
24 hours	24,000	0	0	0	0	0	0

TABLE XII.—*Effect of copper sulfate upon Pseudomonas radicicola (soy).*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	1,900	520	1,300	2,800	650	520	3,100
2 hours	1,800	0	0	45	0	25	1,100
6 hours	2,100	0	0	0	0	0	30
24 hours	18,000	0	0	0	0	0	0

TABLE XIII.—*Effect of copper sulfate upon Bacillus sublanatus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	12,500	780	2,700	2,100	1,100	2,100	1,500
2 hours	12,000	0	0	0	0	35	190
6 hours	12,500	0	0	0	0	0	80
24 hours	40,000	0	0	0	1	0	0

TABLE XIV.—*Effect of copper sulfate upon Micrococcus radians.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	1,200	2,700	10,500	1,700	3,800	2,500	2,800
2 hours	1,200	1	0	1	20	85	10
6 hours	1,400	0	0	0	0	0	1
24 hours	1,900	0	0	0	0	0	0

TABLE XV.—*Effect of copper sulfate upon Pseudomonas radicicola (alfalfa).*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	270	300	330	425	3,700	700	290
2 hours	210	0	0	0	0	0	0
6 hours	220	0	0	0	0	0	0
24 hours	1,175	0	0	0	0	0	0

TABLE XVI.—*Effect of copper sulfate upon Bacillus violaceus laurentius.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	7,200	120	480	740	470	210	520
2 hours	5,300	0	0	0	0	1	25
6 hours	6,800	0	0	0	0	0	0
24 hours	40,000	0	0	0	0	0	0

TABLE XVII.—*Effect of copper sulfate upon Pseudomonas amethystina.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	140	540	90	70	580	670	660
2 hours	190	0	0	1	3	3	15
6 hours	240	0	0	0	0	1	1
24 hours		0	0	0	0	0	0

TABLE XVIII.—*Effect of copper sulfate upon Bacillus caudatus.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	875	60	680	2,100	15	70
2 hours	350	0	0	1	0	0
6 hours	400	0	0	1	0	0
24 hours	9,000	0	0	0	0	0

TABLE XIX.—*Effect of copper sulfate upon Bacillus rubrum.*

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—					
		10,000 parts of water.	25,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	175	10	30	225	90	210	135
2 hours	195	0	0	90	0	0	0
6 hours	50	0	0	5	0	0	0
24 hours	1,050	0	0	5	0	0	0

EFFECT OF CARBON DIOXID ON VIABILITY OF BACILLUS COLI AND BACILLUS TYPHI.

A careful study of the gas content of both tap and triple distilled water has shown that for the typhoid and colon bacilli the presence of carbon dioxid in the water is associated with heightened resistance to toxic agents, such as solutions of copper salts, precipitated copper salts, and copper metal. This is the more strange when one considers that the presence of carbon dioxid in water causes the copper to remain in solution, and in case of insoluble copper, either precipitated or metallic, the carbon dioxid serves to bring a considerable amount of copper into solution. It should be noted, however, that water heavily saturated with carbon dioxid is toxic to *Bacillus coli* and *Bacillus typhi*.

A series of experiments designed to test the effect of carbon dioxid on *Bacillus coli*, the various conditions of triple distilled water with and without copper, triple-distilled water plus calcium carbonate with and without copper, and tap water with and without copper are tabulated below.

TABLE XX.—*Toxicity of copper sulfate to Bacillus coli in the absence of carbon dioxid.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	6,800	1,400	2,300	2,200	900	3,500
2 hours	5,100	0	0	0	2	80

[1] Experiment conducted in Weber resistance glass test tubes each containing 10 c. c. of water triple distilled from glass, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXI.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	9,200	8,200	5,800	11,600	7,500	6,200
2 hours	2,000	0	110	38	900	350

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXII.—*Effect of water containing calcium monocarbonate and various quantities of copper sulfate upon Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,950	3,510	2,670	3,150	4,290	3,760
6 hours	240	0	0	0	105	80
24 hours	5	0	0	0	0	0

[1] Various dilutions of copper sulfate were tubed in Weber resistance glass, thoroughly boiled, a small measured quantity of calcium carbonate added to each tube, and these solutions cooled and kept in a Novy jar in an atmosphere free of carbon dioxid. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry and isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXIII.—*Effect of carbon dioxid content of water containing calcium carbonate and various quantities of copper sulfate upon Bacillus coli.*[1]

Duration of exposure to action of copper suliate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	4,650	4,730	3,850	3,980	4,100	4,470
6 hours	4,500	0	0	5	415	620
24 hours	5,200	0	0	0	15	60

[1] Various dilutions of copper sulfate were tubed in Weber resistance glass, thoroughly boiled, a small measured quantity of calcium carbonate added to each tube, and these solutions cooled and kept in a Novy jar in an atmosphere composed largely of carbon dioxid. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry and isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXIV.—*Toxicity of copper sulfate to Bacillus coli in tap water free of carbon dioxid.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	6,300	7,600	6,000	1,050	4,800	5,200
2 hours	1,500	0	0	0	Lost.	60
6 hours	280	0	0	0	0	0
24 hours	0	0	0	0	0	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXV.—*Toxicity of copper sulfate to Bacillus coli in tap water free of carbon dioxid.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	4,600	1,800	4,800	3,700	4,500	4,000
2 hours	1,450	0	0	20	80	100
6 hours	1,500	0	0	0	5	2
24 hours	250	0	0	0	0	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXVI.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	4,200	4,000	9,800	1,270	5,100	1,100
2 hours	2,800	10	0	0	110	260
6 hours	1,460	15	15	20	10	35
24 hours	1,540	0	0	0	0	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

TABLE XXVII.—*Effect of carbon dioxid upon toxicity of copper sulfate to Bacillus coli.*[1]

Duration of exposure to action of copper sulfate.	Check.	1 part copper sulfate to—				
		10,000 parts of water.	50,000 parts of water.	100,000 parts of water.	500,000 parts of water.	1,000,000 parts of water.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	3,800	3,000	8,500	5,200	4,400	5,500
2 hours	2,300	0	5	15	2,150	1,500
6 hours	1,600	10	12	10	120	100
24 hours	3,500	0	0	10	35	0

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of Potomac tap water, portions of which had been treated previously with the desired amount of copper sulfate. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

An examination of the foregoing tables shows that with the three types of water the presence of carbon dioxid increases the resistance of the bacilli in question. In the solution containing monocarbonate of lime and copper sulfate the bacteria are extremely sensitive, even the bacteria in the check solutions dying rapidly, while in the solutions charged with carbon dioxid the bacteria were able to persist in considerable numbers in the dilute copper solutions in spite of the fact that most of the copper must have remained in solution. This point is interesting in connection with the work of Engels,[a] who reports a

[a] Weitere Studien über die Sterilization von Trinkwasser auf chemischen Wege. Centralbl. f. Bakt., Parasit., u. Infekt., vol. 32 Orig., 1902, pp. 495–521.

rather high toxicity for calcium chlorid, and of Pfuhl,[a] who suggests a calcium salt—milk of lime—for water sterilization. The use of lime or a similar agent may be highly desirable in connection with treating a contaminated reservoir with copper, though the inferior germicidal power of lime makes it improbable that the latter alone could be used safely.

In regard to chemical water analysis, it seems probable that the determination of carbon dioxid or the determination of monocarbonate and bicarbonate alkalinity may have importance hitherto unrecognized, and the variations in the longevity determinations of *Bacillus coli* and *Bacillus typhi* may be due in part to the mineral constituents of a water, in part to methods of experimentation, and in part to the carbon dioxid content. Extended field tests must be made before generalizations on the possible effect of the gas content of a water supply can be determined.

The carbon dioxid content of a water may possibly explain the peculiar results obtained by Clark and Gage.[b] They have reported practically no toxic action from metallic copper, or at least very little difference in the action of metallic copper, iron, tin, zinc, and lead. Their figures are rather misleading because of the great number of days the experiments were carried on, and, as Phelps[c] has shown, metallic copper is coated with some insoluble substance after a few days' exposure to Boston tap water and no longer has great toxic action. The lack of toxicity of metallic copper and the similarity of its action to the action of other metals, as reported by Clark and Gage, is entirely at variance with the work of Kraemer,[d] Pennington,[e] Gildersleeve,[f] Stewart,[g] and Moore and Kellerman.[h] Investigators generally have agreed that it would be possible to practically sterilize

[a] Über die Disinfection der Typhus and Cholera-ausleerungen mit Kalk. Zeitschr. f. Hyg. u. Infekt., vol. 6–s, 1889, pp. 97–104.

[b] The Use of Copper Sulphate in Water Filtration. Journal of Infectious Diseases. Supplement No. 2, February, 1906, pp. 172–174.

[c] Experiments on the Storage of Typhoid Infected Water in Copper Canteens. Public Health Papers and Reports, American Public Health Association, vol. 31, part 1, 1905, pp. 75–90.

[d] Copper Treatment of Water. American Journal of Pharmacy, vol. 76, December, 1904, pp. 574–579.

The Use of Copper in Destroying Typhoid Organisms and the Effects of Copper on Man. American Journal of Pharmacy, vol. 77, June, 1905, pp. 265–281.

[e] The Action of Electrically Charged Copper Upon Certain Organisms in Water. American Journal of Medical Science, vol. 129, 1905, pp. 751–754.

[f] Studies on the Bactericidal Action of Copper on Organisms in Water. American Journal of Medical Science, vol. 129, 1905, pp. 754–760.

[g] A Study of the Action of Colloidal Solutions of Copper upon Bacillus Typhosus, American Journal of Medical Science, vol. 129, 1905, pp. 760–769.

[h] Buls. 64 and 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

drinking water by exposing it to the action of clean metallic copper.[a] Perhaps this opinion should be qualified by adding that the action is more rapid if the water contains no free carbon dioxid, although the following tables show only slight differences between the toxicity of metals because of the presence or absence of carbon dioxid.

TABLE XXVIII.—*Effect of carbon dioxid upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.[2]	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	3,700	1,100	3,300	3,050	3,700	3,500
4 hours	440	75	30	125	275	410
8 hours	305	3	3	30	120	190

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

[2] Iron impure and presumably more toxic than pure iron. (See Table No. XXII.)

TABLE XXIX.—*Toxicity of metals to Bacillus coli in the absence of carbon dioxid.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.[2]	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,950	2,350	1,200	1,450	1,300	1,600
4 hours	965	10	10	65	40	770
8 hours	740	0	0	5	5	700

[1] Experiment conducted in Weber resistance glass test tubes, each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

[2] Iron impure and presumably more toxic than pure iron. (See Table No. XXII.)

[a] There is a seeming discrepancy in this statement due to the fact that Mr. Earle B. Phelps, assistant hydrographer, U. S. Geological Survey, has carried on experiments on the storage of typhoid infected water in copper canteens, some results of which, with conclusions of a nature unfavorable to the use of metallic copper in practically sterilizing water, were issued in a press circular of the Geological Survey. A quotation from Mr. Phelps's paper paralleled with a quotation from Bureau of Plant Industry Bulletin No. 76 shows clearly that Mr. Phelps's work is a corroboration instead of a contradiction of the fact reported in Bulletin No. 76 that metallic copper has a high germicidal value. Mr. Phelps has stated: "The fact that organisms do survive the copper treatment even in small numbers [Per cent reduction usually over 99.999.—K. F. K.] seems, in the writer's view, to lessen considerably the value of the canteen as a safeguard against typhoid infection. A second point of interest is the fact that the efficiency of the canteen decreases as time goes on, probably owing to the accumulation on the surface of the copper of a film of basic carbonate or other insoluble copper compound." A paragraph from Bureau of Plant Industry Bulletin No. 76 reads as follows: "Complete sterilization is a standard to which even the best filters seldom attain, and under the most unfavorable conditions the reduction in the number of bacteria in water exposed to the action of metallic copper for twelve hours will be approximately as great as filtered water. The copper must be kept clean, not, as is popularly supposed, to protect the consumer from copper poisoning, but because it is possible for the metal to become so coated with foreign substances that there is no longer any contact of copper and water, and hence no antiseptic action."

To determine whether the peculiar variation in the germicidal power of solutions or metals is due to the use of ordinary laboratory glassware made of a rather soluble glass instead of carefully selected highly insoluble glass, a parallel series in good and poor glass was carried on. The following tables show the slight difference in results:

TABLE XXX.—*Effect of glass upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,600	1,925	1,850	2,450	2,600	3,100
2 hours	2,650	775	2,100	715	2,800	2,300
6 hours	2,700	525	2,350	70	2,450	4,200
24 hours	28,000	15	4,350	1	13,750	12,000

[1] Experiment conducted in ordinary glass test tubes each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXXI.—*Effect of glass upon toxicity of metals to Bacillus coli.*[1]

Duration of exposure to action of metals.	Check.	Copper.	Iron.	Zinc.	Lead.	Tin.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,300	4,825	4,750	2,400	2,800	3,000
2 hours	1,572	975	2,400	200	1,800	3,600
6 hours	1,400	190	3,050	20	2,000	3,850
24 hours	10,825	1	6,700	1	19,000	14,000

[1] Experiment conducted in Weber resistance glass test tubes each containing 10 c. c. of water triple distilled from glass, to portions of which were added sterile blocks of the proper metals, each having approximately 2 sq. cm. surface area. All tubes inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

COPPER SULFATE AND FILTRATION.

The use of copper sulfate in connection with filtration has been mentioned in previous bulletins. Further experiments in this field show that in mechanical filtration with alum it is necessary to limit the use of copper sulfate to treatment some hours before coagulation. When solutions of aluminum sulfate and copper sulfate are mixed and alkali or hard water is added in quantities sufficient to cause precipitation the copper is coagulated at once, while the aluminum is deposited on the copper and incloses it, with the result that the copper-alum coagulum is no more toxic than is the pure alum coagulum. When copper and iron salts are precipitated together the reverse of this seems to take place and the precipitate retains its toxic properties.[a]

[a] See also H. W. Clark, Sulphate of Alumina as a Germicide. Thirty-sixth Annual Report of State Board of Health of Massachusetts, 1904, p. 288.

The following table shows the various combinations of precipitates tested and the exact results:

TABLE XXXII.—*Toxicity of combined precipitates to Bacillus coli.*[1]

Duration of exposure to action of precipitate.	Check.	Copper hydrate.	Iron hydrate.	Aluminum hydrate.	Copper and iron hydrate.	Copper and aluminum hydrate.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	2,250	1,525	645	7,750	3,450	1,750
2 hours	675	0	230	1,800	25	320
6 hours	465	0	230	195	0	15
24 hours	165	0	230	5	0	1

[1] Experiment conducted in 100 c.c. Jena glass flasks, each containing 15 mg. of the proper precipitate. All flasks inoculated with a 2 mm. loop of culture of *Bacillus coli* received from Prof. Theobald Smith. The temperature during this experiment varied from 18° to 22° C.

TABLE XXXIII.—*Toxicity of combined precipitates to Bacillus coli.*[1]

Duration of exposure to action of precipitate.	Check.	Copper hydrate.	Iron hydrate.	Aluminum hydrate.	Copper and iron hydrate.	Copper and aluminum hydrate.
	Colonies.	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*	*Colonies.*
0 hour	3,350	380	1,065	2,650	3,100	2,350
2 hours	305	0	1,900	3	1	80
6 hours	350	1	1,350	1	0	0
24 hours	200	0	850	1	0	0

[1] Experiment conducted in 100 c.c. Jena glass flasks, each containing 15 mg. of the proper precipitate. All flasks inoculated with a 2 mm. loop of culture of *Bacillus coli* received from the Bureau of Animal Industry, isolated from hog. The temperature during this experiment varied from 18° to 22° C.

The presence or absence of carbon dioxid is probably important in this connection. If the laboratory results will hold for field conditions, a copper precipitate or a precipitate of iron and copper will be highly toxic to *Bacillus coli* and *Bacillus typhi* in a water whose alkalinity is chiefly monocarbonate; and, conversely, the action of a copper precipitate or a copper-iron precipitate will be reduced if a water contains free carbon dioxid. This is probably the reason that small quantities of copper are toxic [a] in a mechanical filter using the proper quantities of iron and copper, and gives an additional reason for the advice given by Ellms [b] and Brown [c] that before filtration or distribution of a copper-treated water all free carbon dioxid and part of the semicombined carbon dioxid should be neutralized by caustic lime. Copper treatment of water previous to slow sand filtration should be made under similar conditions, and as this is seldom practicable it is perhaps advisable to limit the use of copper in connection with slow sand filtration to treatment after passing the filter, and before distribution the proper quantity of caustic lime may be added. An excel-

[a] Bul. 76, Bureau of Plant Industry, U. S. Dept. of Agriculture.

[b] Journal New England Water Works Association, vol. 19, 1905, pp. 496–503.

[c] Journal New England Water Works Association, vol. 19, 1905, p. 578.

lent example of the results to be expected from incorrect use of copper in connection with slow sand filtration is furnished in Clark's[a] report. Not only was copper found in the effluent of the experimental filter, but *Bacillus coli* was found in a rather high per cent of the bacterial samples.

The carbon dioxid content of the Ohio River is a possible explanation also of the fact reported by Brown[b] that samples of Ohio River water yielded between 0.00772 and 0.00657 grain of metallic copper to the gallon, though *Bacillus coli* was occasionally present. To use his own words, "It is seen from the work done, as above stated, that the copper is present in an insoluble form and probably as the oxid united with the suspended mineral matter which the water carries. If in this form it would be unable to exert any germicidal power, and, as far as could be determined, this seems to be the case. It was decided that the copper was present in a spent condition." As there was no monocarbonate alkalinity noted, the Ohio River water at this time doubtless contained some free carbon dioxid, and from the analogy of the laboratory results it seems fair to assume that Brown's explanation must be supplemented by the theory of the heightened resistance of *Bacillus coli* due to carbon dioxid.

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[a] Journal New England Water Works Association, vol. 19, 1905, pp. 503–505.

[b] The Purification of Water at Marietta, Ohio. Official Report to Board of Public Service, 1906.

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VIII.—CONDITIONS AFFECTING LEGUME INOCULATION.[a]

By Karl F. Kellerman, *Physiologist in Charge of Soil Bacteriology and Water Purification Investigations*, and T. R. Robinson, *Assistant Physiologist.*

INTRODUCTION.

The widespread use of bacteria for inoculating leguminous crops has made possible more accurate study of the conditions under which a particular species of legume might be successfully inoculated and the conditions under which failure to obtain inoculation might be expected. Inoculation tests carried on in different soils and under different cultural and climatic conditions but using the same stock culture have shown results so conflicting in certain cases that one is bound to be misled by any general statement based on one factor alone, such, for instance, as the condition of the bacteria used.

To accept as indicative of the general usefulness of pure cultures results obtained on a single type of soil is clearly opposed to progress along a line of investigation now recognized as closely connected with soil fertility problems. The advantage of numerous tests covering many types of soil becomes increasingly apparent. The effect of prevailing cultural practices is no less to be considered; aeration by cultivation, the use of lime, and the effect of such factors on the bacterial flora, the bacteriological testing of the soil itself—all these problems call for extensive field experiments to determine how far they may become part of practical routine for successful farming.

The work here recorded emphasizes the intimate connection of soil bacteriology with certain phases of soil fertility. Soil fertility is a strictly relative term; it is possible for a soil to be fertile in regard to one crop and unfertile in regard to another, the conditions of temperature and moisture being optimum in each case. The interaction

[a] The identification of soil bacteria and their distribution in and correlation with different types of soil, the changes in the bacterial flora with different modes of treatment and different systems of rotation, the individual and combined action of the species in producing changes in the soil favorable or unfavorable to plant growth, the introduction of favorable species and groups of species and their improvement by selection or special cultivation, and the elimination of unfavorable forms are problems calling for extended investigation; but with the information already at hand and work under way both in this country and in Europe it is not too much to expect large increases in our present knowledge along these lines.—B. T. Galloway, *Pathologist and Physiologist, and Chief of Bureau.*

between prevailing soil conditions and biological phenomena becomes apparent at once and lends confirmation to the belief that in practice œcological conditions may be so modified as to promote the activity of the desirable organisms and retard the development of the undesirable, although under certain conditions the control of the bacterial flora undoubtedly will depend upon the introduction of virile cultures of desirable bacteria.[a]

USE OF LIME.

Samples of soil have been obtained from fields in various parts of the United States where attempts to inoculate legumes have failed, and greenhouse tests have been made combining various quantities of air-slaked lime with these soils. The effect of applying lime to certain types of soils is rather striking when nodule formation is considered.[b] This is especially true of those soils which give an acid reaction to litmus,[c] although several of these unfavorable soils did not give an acid reaction to litmus, but were nevertheless benefited by lime. Outlines of a few typical cases selected from our experiments follow.

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[a] The fact as shown in numerous cases that legumes can be successfully inoculated by the use of pure cultures of the nodule-forming organisms with a consequent large gain in yield and amount of nitrogen fixed is in conflict with the statement that "An enhancement of the desirable bacterial activities of the soil can only be encouraged by the proper improvement in the physical and chemical composition of the soil." (Lipman, "The Measure of Soil Fertility from the Nitrogen Standpoint," N. J. Agr. Expt. Sta. Rept., 1905, p. 243.)

[b] Fruwirth. Neue Impfversuche mit Lupinen. Deutsche Landw. Presse, vol. 18, 1892, pp. 18 and 127.

Fruwirth. Dreijährigen Impfversuche mit Lupinen. Deutsche Landw. Presse, vol. 19, 1893, p. 6.

Heinrich. Action de la chaux sur les lupins. Zweit. Ber. Landw. Versuchsst, 1894, p. 272.

Passerini. Sur l'influence améliorante des légumineuses dans des sols de compositions différentes. Staz. Sper. Agr. Ital, vol. 30, 1897, p. 68.

Deherain and Demoussy. Recherches sur la végétation des lupins; deuxieme partie, Lupins bleus. Ann. Agron., vol. 26, 1900, p. 169.

Deherain and Demoussy. Études sur les légumineuses de grande culture. Lupins jaunes. Ann. Agron., vol. 28, 1902, p. 449.

Bilwiller. Cited by Miller. Journal Roy. Agric. Soc. England, 1896, pp. 236 and 423.

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[c] G. A. Billings (N. J. Agr. Expt. Sta. Rept., 1905, p. 358) shows the efficient action of lime in promoting the growth of alfalfa and that the vigor of the plants seemed to be correlated with the form and greater abundance of root nodules. He suggests that soil acidity, conditions of ventilation or soil porosity, and different bacteria which may influence the form of the nodules are determining factors, controlled at least partially by the action of lime.

A quantity of soil was obtained from Blue Hill, Me., where the culture tried last year had shown no benefit with garden peas. One portion of this soil was placed in ordinary greenhouse pots; a second portion was thoroughly mixed with pulverized lime at the rate of 1 ton to the acre, approximately, and placed in similar pots, and inoculated seed sown in half of each series. Of 15 plants unlimed, 10 were without nodules, 5 having a single nodule each; of 17 plants limed, 15 were well noduled and 2 apparently free, but nodules were evident on close examination. The uninoculated pots in the unlimed series had no nodules; in the limed series there were only a few scattering nodules, the majority of the plants, 10 out of 18, being free.

This experiment was repeated, the soil itself being inoculated with the liquid culture, with results even more striking. Of 17 plants unlimed, 12 were without nodules, 5 having a total of 8 nodules; of 16 plants limed, all were well noduled, having a total of 87 nodules. The uninoculated pots in the unlimed series had no nodules; in the limed series, 15 out of 17 plants had no nodules, the other two having a single nodule each.

The combination of liming and inoculation was again strikingly shown with alfalfa grown in a poor sandy soil from Lanham, Md. In this case it seems reasonable to assume that alfalfa bacteria of low virility were present in the soil. Under the normal unfavorable soil conditions the native bacteria were unable to produce inoculation, and the virile ones from the pure culture, while able to inoculate the alfalfa plants, could benefit them only slightly. In the limed soil, however, the native bacteria were able to produce nodules in considerable numbers and to be of moderate benefit to the plants. The pure cultures of virile bacteria under similar conditions, however, caused more than double the increase that may be ascribed to the native bacteria.

The following table shows the relative virility of the two types of alfalfa bacteria in soil from Lanham:

Treatment.	Number of plants.	Number of nodules.	Average height.	Average dry weight.
			Inches.	*Grams.*
Lime:				
Inoculated	9	12	15.5	0.39
Uninoculated	13	15	13	.15
No lime:				
Inoculated	10	11	10	.09
Uninoculated	9	0	9	.06

Similar results were obtained with a large number of soils concerning which unfavorable reports had been received regarding attempts at inoculation with cultures furnished by the Department of Agriculture. Field observations on the nodule formation of various legumes showed the effectiveness of using lime or some other agent

to counteract the influence of substances in the soil unfavorable to the growth of the bacteria.[a]

EFFECT OF SOIL CONDITIONS UPON BACTERIA.

The constitution or character of the soil solution has great effect upon the growth of nodule bacteria as well as upon the formation of nodules. In the large number of soil samples tested simultaneously in the greenhouse and in the laboratory it seems to hold true generally that the possibility or impossibility of securing effective inoculation on a particular crop in a certain soil can be predicted by the relative growth of the specific culture in the sterile extract of the soil in question. There also seems to be a somewhat close relation between the soil solution necessary for the growth of host and bacteria, so that in many cases, at least, it is possible to determine the suitability of a legume crop to a certain soil by the growth of the specific culture of *Pseudomonas radicicola* in the extract of the soil. The probability of an extract not truly representing the soil solution and the disturbing factor of sterilizing certain extracts will in some cases introduce error in interpreting results. The use of sterilized soil extracts as culture media for the bacteria has been found to give fairly true indications of the growth of the respective hosts of these bacteria in a considerable series already tested, however, and contemplated improvements in technique will doubtless increase the probability of securing trustworthy indications. The testing of the soil extract in its behavior toward specific bacteria may even indicate that some treatment is necessary, but can not prescribe the method of amelioration, and for the present direct experimentation is necessary to determine the proper treatment of an unsuitable soil.

As an example of the relation between the growth of the specific bacteria in the sterile soil extract and the growth of the legume host in this soil may be cited the following result of a fairly representative test, using different quantities of lime on an unfavorable soil which responded favorably to treatment with lime:

Quantity of lime.	Green weight of plant.	Number of nodules.	Number of bacteria in a cubic centimeter of soil extract.
	Grams.		
Unlimed	2.5	31	87
Limed:			
2,000 pounds to the acre	5.5	63	71,950
4,000 pounds to the acre	8.5	88	128,650

[a] For the presence of substances in poor soils deleterious to higher plants, see Bureau of Soils Bul. No. 28, 1905, "Studies on the Properties of an Unproductive Soil."

POTS OF RED CLOVER, SHOWING THE EFFECT OF LIME ON A SOIL UNFAVORABLE TO THE GROWTH OF CLOVER.

1.—Unlimed. 2.—Limed at the rate of 2,000 pounds to the acre. 3.—Limed at the rate of 4,000 pounds to the acre.

The accompanying illustration (Pl. VIII) shows characteristic pots of red clover from a series of soils from South Salem, N. Y., where clover had been steadily diminishing in vigor for years. The nodules on the unlimed plants were small and clustered around the crown of the plant, while the limed plants showed numerous nodules well distributed. The stand secured in a series of limed pots was four times as heavy as without the use of lime.

In comparison with this unfavorable soil, the extract obtained from soil sent in from a favorable field test at Carlisle, Pa., gave an excellent growth of the red clover bacteria, and the inoculated red clover grown in this soil in pots showed numerous nodules and a better growth than in the uninoculated pots, thus confirming results obtained in the field.

It may be noted that there is a difference exhibited among different legumes in their behavior toward lime. While for most legumes its effect is beneficial, a few species, such as lupine and serradella, are actually injured by its use[a] and some other legumes, such as Lima bean and cowpea, do not respond to lime even in soils where clover refuses to grow without lime. It has been noted[b] that serradella is not successful when following red clover, and that results are also unsatisfactory when red clover follows serradella. These varying relations shown by legumes belonging to different groups in their relation to lime and other conditions emphasize the necessity of determining as accurately as possible the adaptability of different soils to the crops for which they are intended.

Observations upon the occurrence of nodules in certain soils have been found to correspond quite closely with the behavior of the soil solution toward the bacteria of the legumes tested. The preceding table illustrates this point, though perhaps not so clearly as the following examples.

In the case of a fairly rich garden soil, soy beans would not form nodules even after very heavy inoculation, and the extract from this soil used as a culture medium was found to inhibit the growth of the soy bean bacteria. On the other hand, alfalfa readily formed nodules in this soil and the soil extract was not unfavorable to the growth of the alfalfa bacteria.

A soil from Niles, Mich., which had grown a fair crop of soy beans for four years without the occurrence of nodules, was tested. The soil extract in this case was found unfavorable to the growth of the soy bacteria. In greenhouse pot tests soy beans of several varieties failed to produce any nodules in this soil even when heavily inoculated with cultures which produced abundant nodules in other soils tested

[a] See Rhode Island Agr. Exp. Sta. Rept., 1896, p. 256; also Bul. 96 of same station, 1903.

[b] Deutsche Landw. Presse, vol. 32, 1905, p. 799.

in comparison. Heavy liming of the soil extract at the rate of about 6,000 pounds to the acre gave a fair growth of the bacteria, and corresponding to this result one pot that received lime in the same proportion produced a well-noduled plant. In this same soil without lime and without inoculation cowpeas formed nodules abundantly.

A soil from Lanham, Md., gave an extract which as a culture medium was distinctly unfavorable to the growth of red clover bacteria, and plants of red clover in this soil made a very sickly growth, producing few nodules. A few pots were boiled by immersing in a tub of water and blowing live steam into the water; the soil was of a sandy character, so that there was little danger of puddling from this treatment. The growth of red clover was markedly improved in the boiled series and the roots were well noduled after inoculation. The extract of this soil, sterilized by boiling, had proved an unfavorable medium for the growth of the bacteria. It is probable, therefore, that boiling the soil changed the character of the soil solution at least in regard to the unfavorable materials or conditions which had inhibited bacterial activity.

Soil from Lanham, Md., was further tested with alfalfa, treating the soil in three ways: (1) Lime at the rate of one ton to the acre; (2) lime and one-fourth humus, composed of leaf mold; (3) humus. The combination of lime and humus gave the greatest growth and most abundant nodule formation. With humus alone the growth was especially inferior and nodules were lacking even where the soil was inoculated. Inoculation doubled the number of nodules where lime was used and increased them six times where both lime and humus were used.

Effect of humus and lime upon the growth of alfalfa in soil from Lanham, Md.

[Ten plants in each series.]

Treatment.	Average (green) weight of plant.	Average number of nodules.
	Grams.	
Lime alone:		
Uninoculated	0.86	0.5
Inoculated	1.56	1.0
Humus alone:		
Uninoculated	.5	0
Inoculated	.8	0
Humus and lime:		
Uninoculated	.73	1.4
Inoculated	2.45	8.6
No lime; no humus:		
Uninoculated	(*)	.3
Inoculated	(*)	.5

*Not weighed; growth so small as to be a practical failure—2 to 5 inches as compared with 12 to 18 inches high in the limed pots or those containing lime and humus.

The sterilized extracts of soil from this series of pot tests gave results seemingly contradictory when used as culture media for bacteria in comparison with the growth of plants and nodules. The

extract of untreated soil gave a growth of the alfalfa bacteria which might be designated "fair," while with lime, with lime and humus, and with humus alone the extracts gave uniformly a growth which might be designated "excellent." In the pots, however, no nodules developed in the humus series. The sterilization by heat of the extract from a soil containing such a relatively large quantity of humus, one-fourth by volume, probably changes the material so that the solution becomes favorable to the growth of the bacteria, but this material in the soil pots, not heated, remains unchanged and may in this form be unfavorable to the activity of the bacteria introduced into the soil.

Aside from the effect of the soil on nodule formation there seems to exist a marked difference among different varieties of legumes in their susceptibility to infection—that is, their readiness to form nodules. With a similar soil and one which is favorable to the growth of the respective species, nodules will occur in abundance upon one species or variety and another species or variety will exhibit none or only a few. This is particularly noticed with varieties of soy beans and with certain species of Phaseolus, the Lima bean (*Phaseolus lunatus*) and some varieties of soy beans being very difficult to inoculate. It would seem that such species were actually resistant to infection, especially in soils rich in nitrogenous matter.

EFFECT OF HEAVY INOCULATION.

The decided advantage of very heavy applications of pure cultures reported from some field and pot experiments and reports of other experiments showing that cultures diluted to almost infinitesimal limits gave as good results as undiluted cultures seem at first diametrically opposed. Greenhouse tests seem to confirm the beneficial effect to be expected from heavy inoculation provided excessive quantities of culture are applied. The following experiment is fairly representative:

Effect of light and heavy inoculation of garden peas on soil from Blue Hill, Me.

Treatment.	Number of plants grown.	Number of plants with nodules.	Average number of nodules.
Check, without inoculation	11	2	1
Light inoculation	9	6	3
Heavy inoculation	6	6	7

It is here shown that heavy inoculation not only doubled the average number of nodules upon inoculated plants, but insured all plants becoming infected; while with light inoculation in this unfavorable soil one-third of the plants failed to produce nodules, and with no inoculation nodules were almost entirely lacking.

Garden peas in a garden soil naturally inoculated showed the following difference where a heavy inoculation was made:

Effect of heavy inoculation of garden peas on a naturally inoculated garden soil.

Treatment.	Number of plants grown.	Number of plants with nodules.	Average number of nodules.
Untreated	15	15	6½
Heavy inoculation	18	18	19

It is highly probable that the cause of the formation of more numerous nodules in some cases is due not to the great numbers of bacteria which are introduced into the soil but to the quantity of culture medium introduced, which renders the soil solution a materially richer food for the active growth of the nodule organisms. Thus, if a few bacteria are brought into a favorable soil they will multiply rapidly, and it is of no particular moment in such a case whether a very few or a large number of bacteria are originally introduced. If, however, enough culture is added so that the soil solution is appreciably improved in food material for both plant and bacteria, then growth will be much more active and nodule formation will be greater. On this hypothesis the two results are not at all at variance, the effects produced depending on the soil conditions encountered by the bacteria and the crop.

The suggestion in a former bulletin[a] that the slight varietal characteristics exhibited by legume bacteria can be readily broken down by cultivation on synthetic nitrogen-poor media seems to hold only for bacteria isolated from plants physiologically related to the subsequent hosts.[b]

A typical experiment is given below:

Number of nodules produced on two varieties of mung bean (Phaseolus viridissimus and P. calcaratus) by cultures from various hosts.

[Ten plants in each of seven series.]

Variety of mung bean.	Bacteria from cow-pea.	Bacteria from soy bean.	Bacteria from P. viridissimus.	Without bacteria.
Phaseolus viridissimus	*1	0	77	0
Phaseolus calcaratus	0	0	88	0

*Fifteen plants in this series.

It may be added that the soil used was unsterilized garden soil, naturally inoculated for our common bean, *Phaseolus vulgaris*, so that the uninoculated check may be said to serve as a cross-inoculation with these organisms also. This theory of varietal differences of nodule-

[a] Bul. 71, Bureau of Plant Industry, 1905, pp. 25–27.

[b] See Schneider, Ill. Agr. Exp. Sta. Bul. 29, 1894, p. 301.

FIG. 1.—POTS OF GARDEN PEAS, SHOWING THE EFFECT OF AERATION ON GROWTH.

1.—Ordinary unglazed pot; surface of soil frequently stirred. 2.—Glazed pot; surface of soil paraffined and water introduced through separatory funnel.

FIG. 2.—THE GARDEN PEAS ILLUSTRATED IN FIGURE 1, SHOWING THE EFFECT OF AERATION ON NODULE FORMATION.

1.—Plant from unglazed, aerated pot. 2.—Plant from glazed pot from which air was partially excluded.

forming bacteria is closely in accord with field results reported by various investigators, among whom perhaps Hopkins[a] is best known for his observations upon the cross-infection between sweet clover and alfalfa and the absence of such cross-infection between many other legumes.

EFFECT OF AERATION.

The aeration of soil for securing plentiful nodule formation is an important if not a determining factor. The Ontario Agricultural Experiment Station (Report for 1905, p. 39) reports a decided gain from aeration in the case of a leguminous crop (peas) and no gain from the aeration of a wheat crop. In the former case the result may be either a direct one, affecting the activity of the nodule bacteria, or it may be that because of the abnormal growth of the host plant the bacteria are unable to penetrate the root.[b]

Our own experiments upon the effect of aeration are in accord with those quoted in regard to the legumes, and the following is typical: A light sandy soil, moderately limed, was used and the legume selected was the garden pea. The aerated series was started in ordinary unglazed 4-inch pots, seedlings dipped in liquid culture when placed in position, pots watered with a fine sprayer, using tap water. The surface of the soil was frequently stirred. The nonaerated series was made up of an equal number (6) of glazed 4-inch pots with bottoms plugged with paraffined cotton and the tops covered with paraffined paper to reduce aeration. A separatory funnel (shown in Pl. IX, fig. 1) was thrust into each of these pots to admit water for growing the peas, and after inoculating as with the other series the seedlings were introduced through

[a] Ill. Agr. Exp. Sta. Bul. 94, 1904, Nitrogen Bacteria and Legumes.

[b] Livingston (Bot. Gaz., XLI, 2, 1906, p. 143; see also Bureau of Soils Bul. No. 28) reports a close relation between the growth of roots and tops in wheat, due to a difference of soil, which he explains as follows: "It would seem that the poor soil by inhibiting branch growth and causing the enlargement of cortical cells may render the root system unable to carry on an adequate amount of absorption for normal growth." If this effect upon the cortical cells should be found to hold true with the legumes, it might have a direct bearing upon the ability of the bacteria to penetrate and form nodules. The enlargement of the cortical cell is regarded as a phenomenon of age, and it is reported (Maria Dawson, Phil. Trans. Roy. Soc. London, Ser. B, vol. 192 (1899), p. 24) that infection takes place most readily in quite young radicals.

Aside from the effect of root development upon nodule formation, there is a possible interaction resulting in some way in greater root development where inoculation takes place. In many cases which have come to notice where inoculated plants had shown a superior development of the root system, it seemed at least justifiable to regard the inoculation as assisting in providing the conditions favoring a healthy root growth. In Bulletin No. 237 of the Cornell Agricultural Experiment Station, page 165, it is noted that where no nodules occur the roots of alfalfa are simpler and not so ramifying as where nodules occur.

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a hole in the paraffined paper. After a month's time the roots of the plants were examined, with the following results:

Treatment.	Nodules on plant No.—						Total.
	1.	2.	3.	4.	5.	6.	
Aerated	4	20	25	8	26	4	87
Nonaerated	0	1	2	0	0	0	3

See Plate VIII, figure 2, noting the difference in the plants in favor of the noduled (aerated) plant.

ASSOCIATIVE ACTION OF BACTERIA.

Undoubtedly the pure cultures of nodule-forming bacteria are subject to contamination when planters, using convenient rather than bacteriological methods, prepare quantities sufficient to inoculate their seed. Yet the purity of a culture immediately before it is applied to seed or introduced into the soil of a cultivated field is unimportant, provided there is present a sufficient number of virile nodule-forming bacteria.

Preliminary experiments at Washington some years ago indicated that in the nitrogen-poor solution generally used the nodule-forming bacteria would develop much more rapidly than other bacteria or contaminating yeasts and molds. It might be expected that different localities would have different contaminating forms, and during the past year samples of cultures have been obtained from farmers in various parts of the United States immediately before the seed or soil was treated. Investigation of contaminating forms isolated from these cultures shows that there are certain forms which inhibit the growth of the nodule organism when both are grown for some time in the usual nitrogen-poor solution.[a] Using an extract of a favorable soil as a culture medium, results very nearly parallel for the competing bacteria were secured, though one organism which resembles *Bacillus coli* checked the growth of *Pseudomonas radicicola* in the synthetic medium and not in the soil extract. The relative virility of the competing cultures at the time they begin their struggle is an exceedingly important factor in determining which species of bacteria survives.

Extended experiments on the interaction of groups of bacteria in various media must be carried on before it will be possible to deter-

[a] In view of this fact, the reason for our present method of distribution is apparent. A relatively large amount of pure liquid culture used as a "starter" cuts down the chances of ruining the farmer's culture through the competition of contaminating forms; the time required to fill the solution with the organisms is shortened, and, as shown subsequently, the quicker the bacteria can be introduced into the soil and make use of the soil solution as a culture medium the less becomes the danger from competition.

mine all of the conditions incident to success or failure in legume inoculation.[a] It is safe to assume, however, that the action of a bacterium in the soil is conditioned not only by the chemical and physical characteristics of the soil solution at a particular time, but also, and perhaps essentially, by the biologic conditions obtaining in that soil.

SUMMARY.

1. Lime is of decided benefit in obtaining successful inoculations of legumes in some soils. These soils often show an acid reaction to litmus.

2. Soil extracts serving as culture media often indicate the probable success of inoculating a leguminous crop. This, however, may not always hold true.

3. At least during the first season's growth no general cross-inoculation takes place. Bacteria from one host may, however, inoculate a physiologically related species.

4. Heavy inoculation by a pure culture increases nodule formation if the soil solution is enriched by the excess of culture medium; however, in a favorable soil a light inoculation well distributed is as effective.

5. Thorough aeration is favorable to nodule formation.

6. Whether in a synthetic medium or a natural soil solution, the functions of a bacterium are influenced by the associative or competitive action of the various groups of organisms with which it comes in contact, as well as by the nature of the culture material.

[a] See Maria Dawson, Phil. Trans. Roy. Soc. London, Ser. B, vol. 193 (1900), p. 65.

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